CHEMISTRY OF NATURAL PRODUCTS

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A C K N O W L E D G E M E N T

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C O N T E N T S

T H E O R E T I C A L

I N T R O D U C T I O N

Flavones with their kindered, form the largest single family of naturally occurring oxygen heterocycles: as plant pigments, they are exceeded in importance only by anthocyanins, chlorophyll and carotenes. The close connection with other members of the C_6-C_3-C_6 fraternity e.g., anthocyanidins, catechins and aurones further enhances the importance of this group. The flavones rely for their numerical impressiveness less on structural diversification than on the manner in which the parent nucleus is hydroxylated and alkoxylated: indeed a full description of more than a few individual flavones just amounts to excessive repetition. Numerous physiological activities have been attributed to these compounds but many of these claims have been strongly disputed, and of the remainder few are important. The potent uses of flavonoids may be listed as Vitamin P activity (i.e., the property of reducing the capillary fragility and permeability); diuretic and anthelminthic effects; prevention of amphylactic shock, protection against X-rays and other radiation injuries; cure of frostbite; hypotensive, hypertensive, bacteriostatic and bactericidal activity; as cardiac stimulant and vascoconstrictors; estrogenic activity; and antitumor effects.

Some years back, the phenolic extractives of the autumn leaves of maiden hair tree (Ginkgo biloba) were shown to increase the flow of blood in cerebral and peripheral areas. Detailed studies carried out by Nakazawa[1] and Kawano[2] (in Japan) and Baker[3] (in U.K.) have disclosed the existence of several related flavonoid pigments in these extracts. The fundamental nucleus in these is one in which the two flavone (C_{15}) units are linked together forming a new type of flavonoid pigments - Biflavonoids. Following upon this, considerable study has been made mainly in Japan and India, of related plants (Gymnosperms) and many biflavonoids have been discovered.

The naturally occurring biflavonyl compounds may be classified into two main groups:-

A. C-C linked biflavonyls

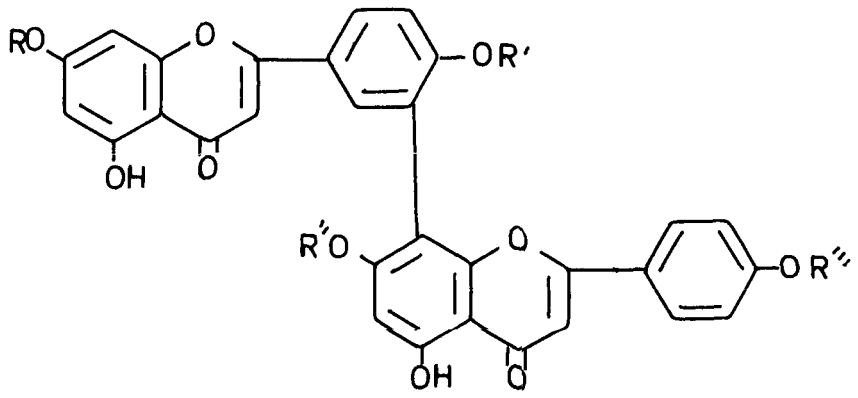
B. C-O-C linked biflavonyls

The C-C linked biflavonyls may be further divided as A_1, A_2, A_3 & A_4.

A_1 Type

These are derived from apigenin with 3'-8" linkage and are represented by amentoflavone[4] as the parent compound. There are eleven partial methyl ethers of this series known

todate.

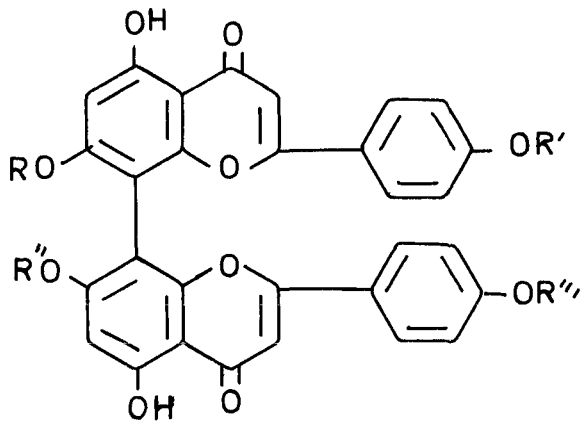


(I)

		R	R'	R''	R'''
(a)	Amentoflavone	H	H	H	H
(b)	Sotetsuflavone	H	H	Me	H
(c)	Bilobtin	H	Me	H	H
(d)	Sequoiaflavone	Me	H	H	H
(e)	Podocarpusflavone A	H	H	H	Me
(f)	Podocarpusflavone B	Me	H	H	Me
(g)	Isoginkgetin	H	Me	H	Me
(h)	Ginkgetin	Me	Me	H	H
(i)	Kayaflavone	H	Me	Me	Me
(j)	Sciadopitysin	Me	Me	H	Me
(k)	Heveaflavone	Me	H	Me	Me
(l)	4', 4''', 7, 7''-Tetra-O-methyl amentoflavone	Me	Me	Me	Me

A_2 Type

These are derived from apigenin but with 8-8" linkage. This class of biflavones is represented by four members. Cupressuflavone[5] is the parent compound while the other three are its partial methyl ethers.



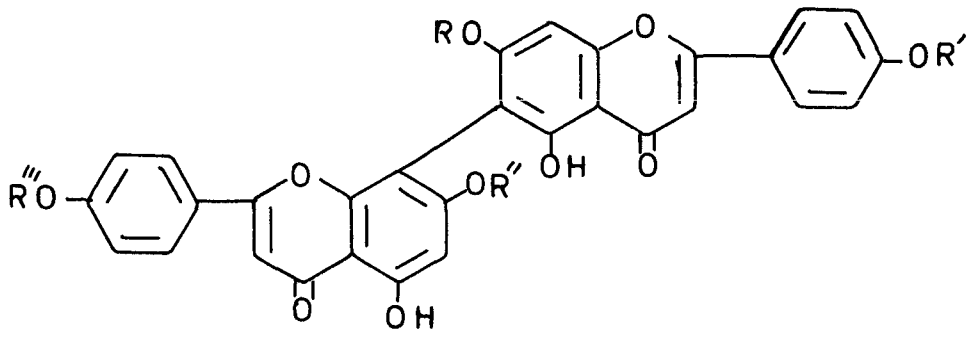
(II)

		R	R'	R"	R"'
(a)	Cupressuflavone	H	H	H	H
(b)	7-O-Methyl cupressuflavone	Me	H	H	H
(c)	7,7"-Di-O-methyl cupressuflavone	Me	H	Me	H
(d)	4', 4"', 7,7"-Tetra-O-methyl cupressuflavone	Me	Me	Me	Me

A_3 Type

These are derived from two apigenin units with 6-8" linkage. This class of compounds has been recognised very

recently and comprises of three partial methyl ethers[6]. The parent compound has not been reported so far.

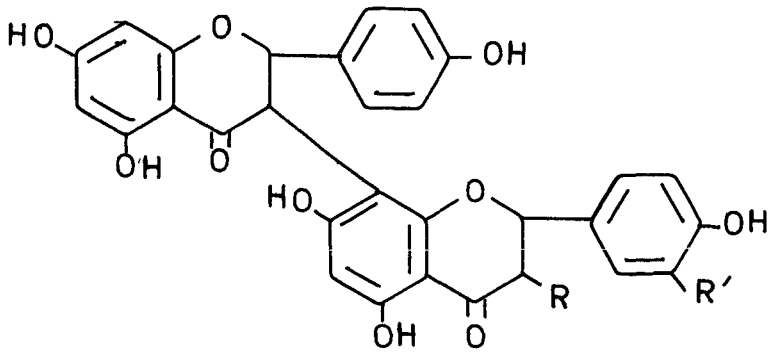


(III)

		R	R'	R''	R'''
(a)	Agathisflavone A	Me	H	H	H
(b)	Agathisflavone B	Me	H	H	Me
(c)	7, 7''-Di-O-methyl agathisflavone	Me	H	Me	H

A_4 Type

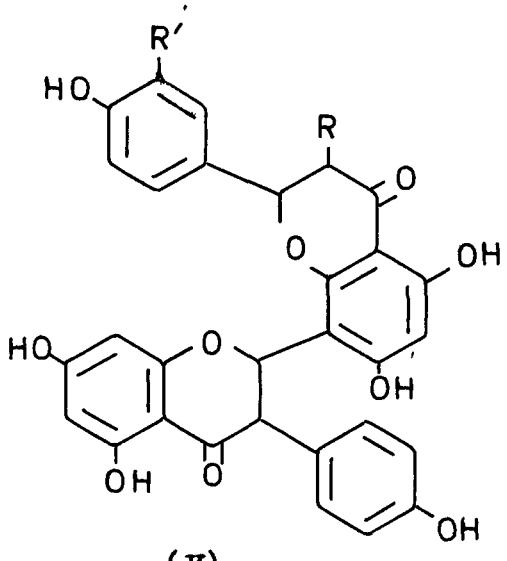
This class comprises of fully or partially reduced heterocyclic systems. Seven members are known todate. Four members of GB series[7] are derived from naringenin linked with naringenin or aromadendrin, or taxifolin through 3-8'' linkage.



(IV)

		R'	R"
(a)	GB-1	OH	H
(b)	GB-1a	H	H
(c)	GB-2	H	OH
(d)	GB-2a	OH	OH

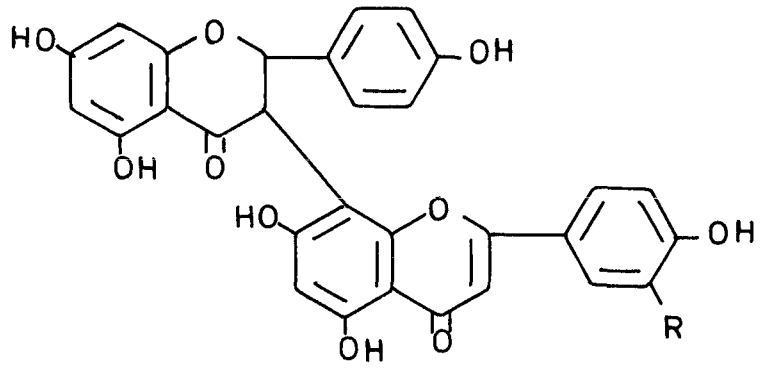
Pelter[8] has proposed an alternative structure for the above series. According to him the linkage should be 2-8" (i.e. isoflavanone-flavanone).



(V)

		R'	R"
(a)	GB-1	OH	H
(b)	GB-1a	H	H
(c)	GB-2	H	OH
(d)	GB-2a	OH	OH

The remaining two members[9,10] are derived from flavanone-flavone units linked through 3-8".



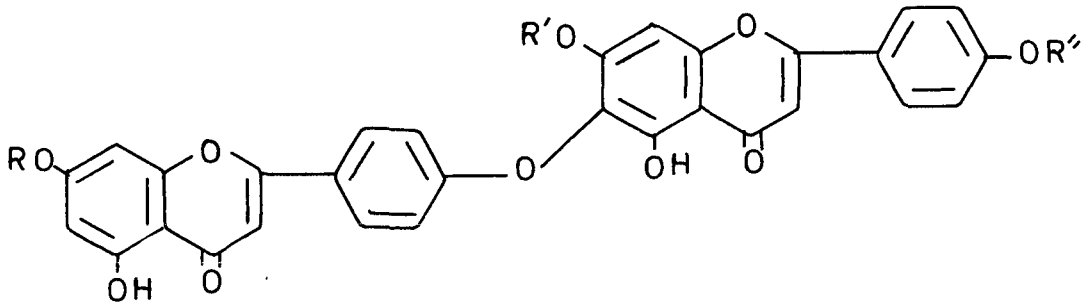
(VI)

(a)	Morelloflavone	R = OH
(b)	3"'-O-Methyl fukugetin	R = OMe

Whereas morelloflavone is optically inactive, its optically active form has been isolated from Garcinia spicata very recently. The optically active form has been named 'fukugetin'[10].

B Type

Seven natural biphenyl ether type of biflavonyls have been reported so far. These include hinokiflavone as the parent compound[11]. The remaining six are its partial methyl ethers. They involve 4'-O-6" linkage between two apigenin units.



(VII)

		R	R'	R"
(a)	Hinokiflavone	H	H	H
(b)	Cryptomerin A	H	H	Me
(c)	Isocryptomerin	H	Me	H
(d)	Neocryptomerin	Me	H	H
(e)	Cryptomerin B	H	Me	Me
(f)	Chamaecyparin	Me	H	Me
(g)	4"', 7, 7"-Tri-O-methyl hinokiflavone	Me	Me	Me

STRUCTURE DETERMINATION OF BIFLAVONOIDS:

The problem of structure determination of biflavonoids is a complex one because of (a) inherent difficulties experienced in their isolation in pure and crystalline forms (b) insolubility in the usual organic solvents and (c) the intricate problem of establishing the interflavonyl linkage.

The various methods generally used for structure determination may be classified as under:

1. Colour reactions
2. Physical methods
3. Degradation
4. Synthesis

1. COLOUR REACTIONS

A number of colour reactions are reported in literature for detecting certain structural features among flavonoids. As the colour depends upon the pattern of hydroxylation and substitution, the diagnostic value of colour is only a broad indication. Biflavonoids are found to give more or less the same colour reactions as monomers. The reagents generally used for colour reactions are magnesium-hydrochloric acid[12], sodium amalgam-hydrochloric acid[13], Wilson boric acid[14] and zinc-hydrochloric acid[15].

2. PHYSICAL METHODS

Chromatographic and spectral methods (i.e., IR, UV, NMR and Mass spectrometry) have been applied in the identification and structural analysis of plant pigments. They provide an excellent tool in the hands of organic chemist for elucidation of structure of even minor compounds.

CHROMATOGRAPHIC METHODS

A number of papers and review articles have appeared on the separation and identification of flavonoid pigments especially by paper chromatography in aqueous and alcoholic solvent systems[16-19]. In the case of biflavonoids, however, this technique has not been much successful because of their high mobility, very close R_F values and indistinguishable colour shades[20]. Thin-layer chromatography has been used by Kawano et al[21] for the detection of the biflavone constituents of 12 gymnosperms using toluene:ethyl formate:formic acid (5:4:1) as developing system. Some reports on the use of column chromatography for the quantitative separation of pigments from crude extracts have also appeared[9,10]. The most successful method for quantitative separation at the moment is counter current distribution between ethyl methyl ketone and borate buffer (pH 9.8)[3]. Recently the use of dry column chromatography for quantitative separation has also been reported[22].

ULTRAVIOLET SPECTROSCOPY

The position of maximal absorption of biphenyl type biflavones belonging to amentoflavone, agathisflavone, cupressuflavone and their derivatives are very similar to that of apigenin and its derivatives, with the only difference that the molecular extinction coefficients of biflavones are approximately double as compared to the corresponding monomers. This demonstrates the presence of two isolated chromophores of flavonoids per molecule of biflavonyls. The effects of the diagnostic reagents such as N/50 NaOEt, N/500 NaOEt, N/5000 NaOEt, NaOAc and $AlCl_3$ on the spectra of biflavonyls are similar to those in monomers. The difference in activity of hydroxyl groups may arise due to steric factors. These differences have been well exploited by Baker et al[3] in assignment of methoxy groups in isoginkgetin and ginkgetin, kayaflavone and sciadopitysin etc.

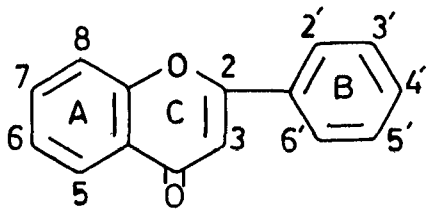
NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

The application of NMR spectroscopy has proved to be the most powerful tool in structure determination of flavonoids. It has been possible now to determine the structures of flavonoids occurring even in minor quantities solely on the basis of NMR studies without tedious and time consuming chemical degradation and synthesis.

The observation and interpretations of spin-spin splittings are the means by which the sequence of groups

in molecules is established by NMR. However, the process of establishing sequences of groups in molecules, even on high resolution NMR, frequently fails, because, while it may be possible to observe a discrete multiplet from one group of protons it may be impossible to recognise the absorptions of protons to which this group is coupled, since they may be obscured by absorptions of other protons in the molecule. An ancillary technique known as spin-decoupling, double resonance or multiple resonance often helps to overcome this difficulty. By the help of double irradiation technique it has been possible to assign each and every proton in biflavonyls. The recent technique of preparing silyl derivatives for NMR studies has not only overcome solubility problem but also has contributed towards the simplification of spectra[24a,b].

The valuable contributions in the field are by Batterham and Highet[23] Mabry et al[24], Massicot et al[25], Clark-Lewis et al[26] and Kawano et al[11]. In flavonoids the chemical shifts of the protons of rings A and B prove to be independent of each other but are affected by the nature of ring C[23]. The peaks arising from ring A in most flavonoids occur upfield from the other peaks and are readily recognized. Of the substitution patterns encountered in the ring A of



naturally occurring flavonoids, the 5,7-dihydroxy or phloroglucinol residue is by far the most common. In flavanones the 6,8-protons give a single peak near τ 4.05; with the addition of a 3-hydroxy group (flavanonols) the chemical shifts of these protons are slightly altered and the pattern changes to a very strongly coupled pair of doublets. The presence of the double bond in ring C of flavones and flavonols causes a marked downfield shift of these peaks, again producing the two doublet pattern. Out of 6-, and 8-protons the latter appears downfield.

The signals from the aromatic protons of an unsubstituted ring B in a flavanone appear as a broad peak centered at about τ 2.55. In flavones, presence of the ring C double bond causes a shift of the 2', 6' protons and the spectrum shows two broad peaks, one centered at τ 2.00 (2', 6') and the other at τ 2.4 (3', 4', 5').

With the introduction of a 4'-hydroxyl group, the ring B protons appear effectively as a four peak pattern. The OH group increases the shielding on the adjacent 3', 5' protons and their peaks move substantially upfield. The 2', 6' protons of flavanones give signals centered at about τ 2.65. Introduction of the 2,3 double bond (flavones and flavonols) again causes these protons to resonate at much lowerfield. Such type of pattern is called A_2B_2 pattern. Introduction of one more substituent to ring B gives the

normal ABC pattern.

In the spectra of flavones and isoflavones of normal structure the olefinic protons give rise to signals near τ 3.2 and τ 1.7 respectively. It has been observed that the position of these olefinic peaks also depends upon the substitution of the rings A and B, the electron donating groups causing upfield shifts and electron withdrawing groups causing downfield shifts.

The spectra of flavanones (saturated heterocyclic ring) contain typical ABX multiplets arising from the 2-proton and the two 3-protons. The 2-proton is generally a double doublet near τ 4.5, the precise position depending on the substitution of ring B, while the protons at the 3-position give rise to a multiplet of eight lines near τ 7.0. 3-Hydroxyflavanones give rise to a AB quartet in the region τ 4.4 - 5.6 with a characteristic coupling constant of 12 c/s.

The relative stereochemistry of 3-substituted flavanones can usually be established from a consideration of vicinal coupling constants and the "KARPLUS equation". In all cases, the heterocyclic ring appears to adopt the chair or half chair conformation in which 2-aryl substituent is quasi-equatorial.

Massicot and Marthe[25], analysing the ABX spectrum of heterocyclic ring protons of 6,7-dimethoxy flavanone, have

shown the two vicinal coupling constants to be 13.5 and 3.2 c/s The former is clearly a diaxial interaction, thus establishing the equatorial character of 2-aryl group in flavanones. All 3-hydroxy and 3-acetoxy flavanones, which have been examined, exhibit vicinal coupling constants 12 c/s and were therefore assigned the trans (di-equatorial)-configuration, although in the case of naturally occurring compounds the possibility of epimerization can not be excluded.

The proton of a 5-hydroxyl group next to a 4-carbonyl of a flavonoid gives rise to a sharp signal at a very low-field consistent with the strong hydrogen bonding between the two groups.

Methylation of a hydroxyl group commonly produces an upfield shift ($\sim$ 0.2 ppm) of the signals of ortho protons with a somewhat smaller effects on those of para protons and little or no effect on the meta protons. Acetylation of the hydroxyl group, as expected, causes downfield shift of the ring protons.

In amentoflavone, agathisflavone and cupressuflavone series, the biphenyl type of biflavones, the peaks of ring protons involved in interflavonyl linkage appear at somewhat lowerfield ($\sim$ 0.5 p.p.m.) (as compared to chemical shifts of the same protons in monomers) due to extended conjugation.

It has been observed both in biphenyl as well as biphenyl ether type series of biflavonyls that the 5-methoxyl

group in 8-8" linked biflavones or in 8-linked monoflavonoid unit of a biflavone shows up below τ 6.0 in deuterochloroform[6] (Table I).

T A B L E - I

Biflavonoids	5-OMe	5"-OMe
1. Cupressuflavone hexamethyl ether	τ 5.85	τ 5.85
2. Amentoflavone hexamethyl ether	τ 6.13	τ 5.94
3. Agathisflavone hexamethyl ether	τ 6.41	τ 5.95
4. Hinokiflavone pentamethyl ether (4'-O-8")	τ 6.00	τ 5.92
5. Hinokiflavone pentamethyl ether (4'-O-6")	τ 6.06	τ 6.09
6. GB-1 heptamethyl ether	above	τ 6.04
7. Fukugetin (Morelloflavone)-heptamethyl ether	above	τ 6.07

This observation may be explained on the basis of extended conjugation in the first four compounds.

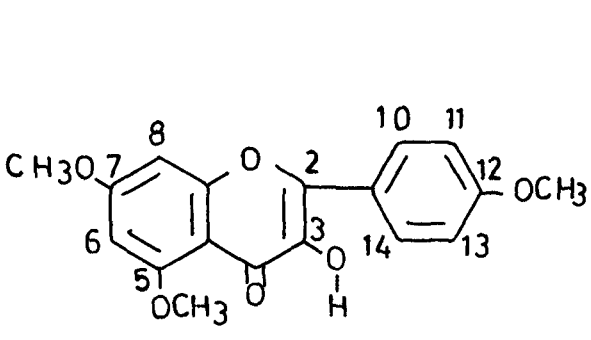
The observation of chemical shift of one of the methoxy groups of the 4'-O-6" hinokiflavone pentamethyl ether at τ 5.92 as reported by Kawano et al[11] led us to believe that, perhaps, the hinokiflavone must be 4'-O-8". On running the NMR spectra of 4'-O-6" and 4'-O-8" hinokiflavone pentamethyl ethers in pyridine, the solvent used by

Kawano et al[11], it was found that in confirmation with previous findings and contrary to our expectations, the 5"-methoxy group of 4'-O-6" linked hinokiflavone pentamethyl ether shows up at τ 5.88 (below 6.0) and that of 4'-O-8" linked biflavone above τ 6.0. This discrepancy was explained by the consideration of influence of solvent on chemical shift[27].

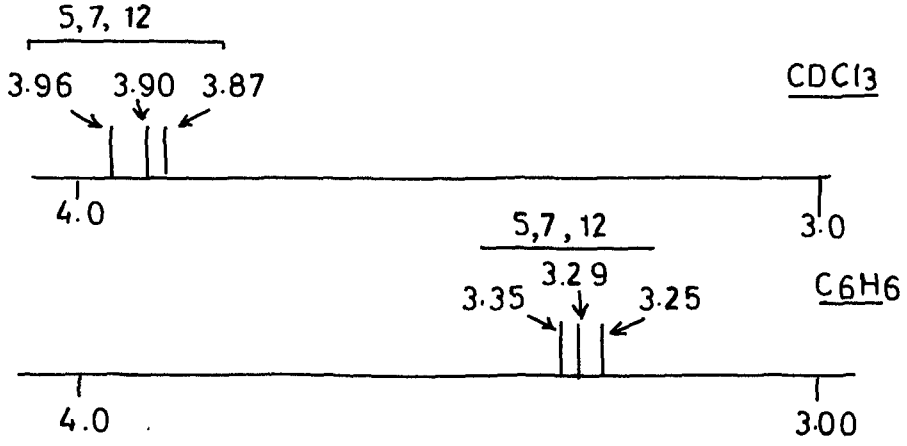
Recently the solvent dependence of methoxy resonances induced by benzene (relative to comparatively inert solvent, such as CCl_4 or $CDCl_3$) upon electronic, steric and conformational factors has been noted[28-31].

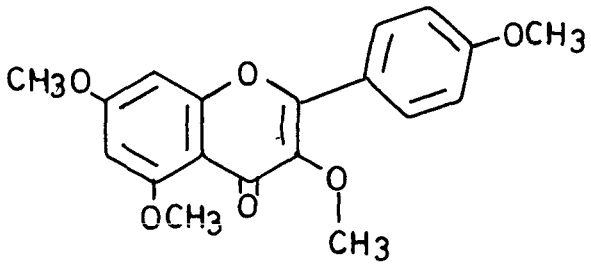
The position and relative orientation of methoxy groups in methoxy flavones can be inferred from benzene induced solvent shifts of methoxy resonances[32]. From the consideration of the results in toto (Chart I), it is apparent that if the local environment (mainly with regard to immediately adjacent substituents) of a methoxy group is defined, the solvent shifts are characteristic of local environment and frequently characteristic of the position of the substitution.

CHART - 1



(VIII)

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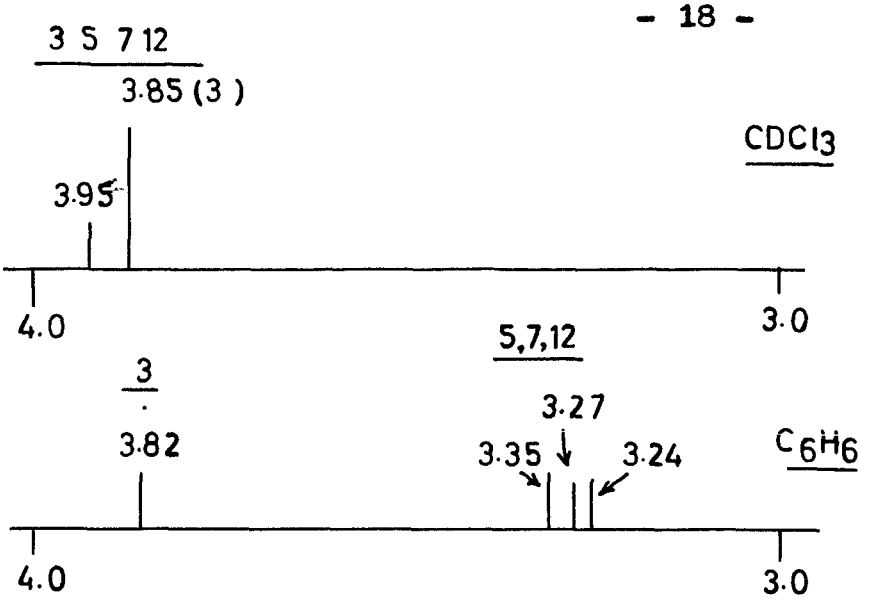
O
OCH3
O
O
CH3

(IX)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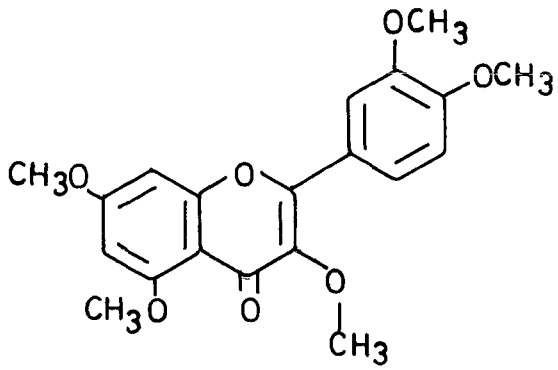
CDCl3
3.95
4.0
3.0
5,7,12
3
3.27
3.82
3.35
3.24
C6H6
4.0
3.0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CH3O
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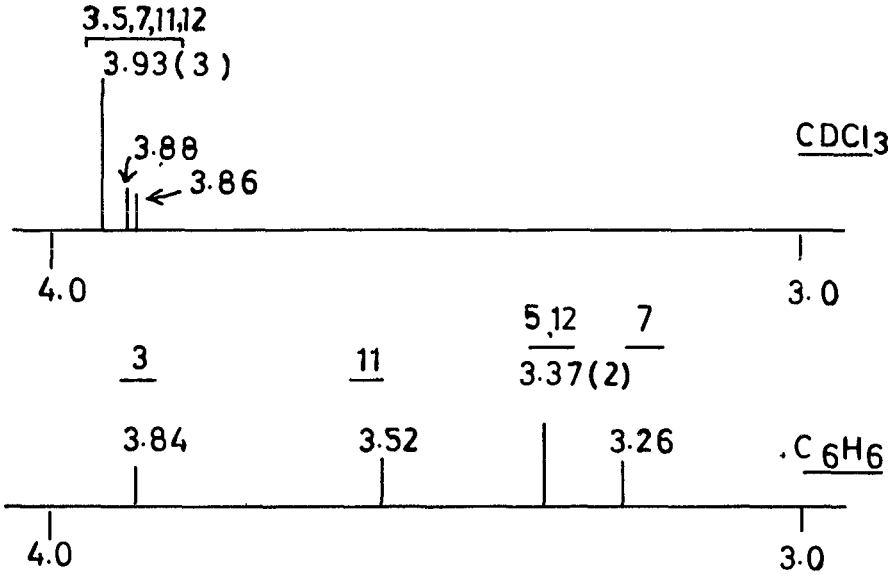
O
CH3O
O
CH3

(X)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5,12
7
3
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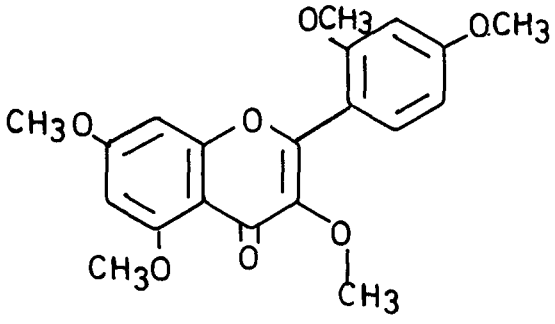
3.0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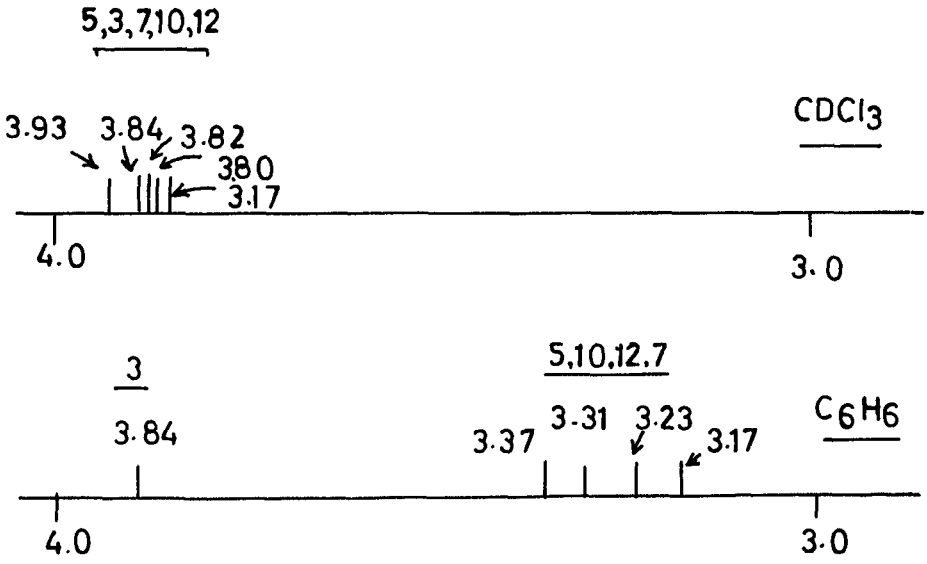
OCH3
CH3O
O
O
CH3O
O
CH3

(XI)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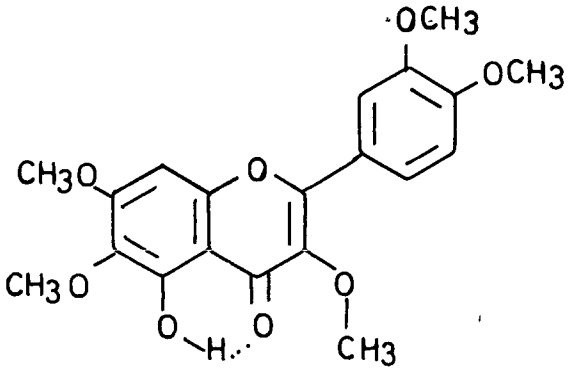
3.23
3.17
C6H6
4.0
3.0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CH3O
O
O
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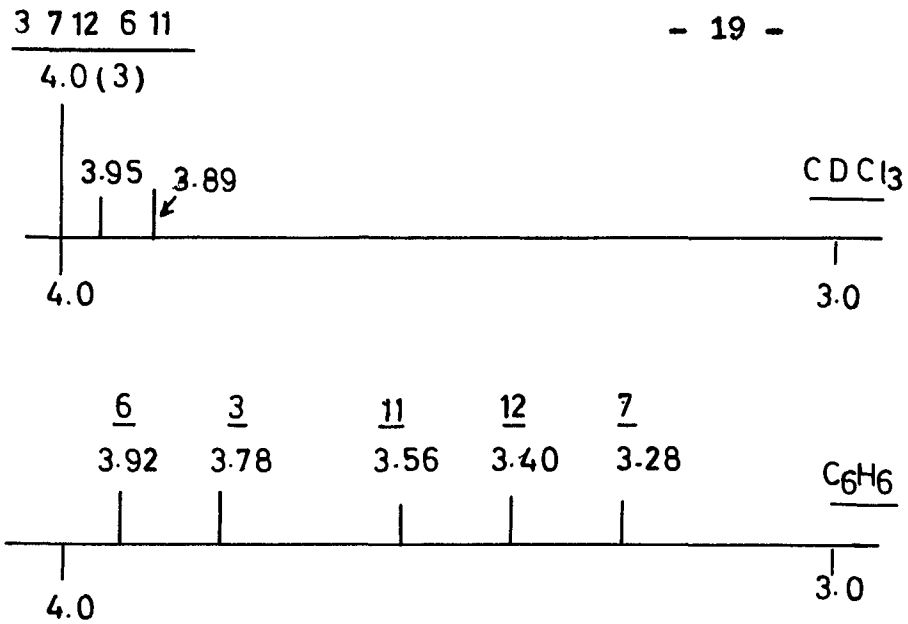
H
O
CH3

(XII)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3.95
3.89
CDCl3
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3.0
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3.40
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C6H6
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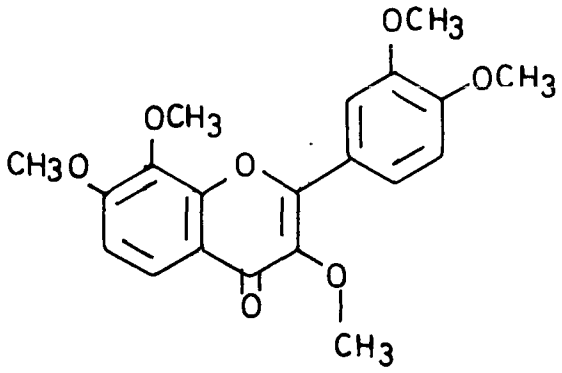
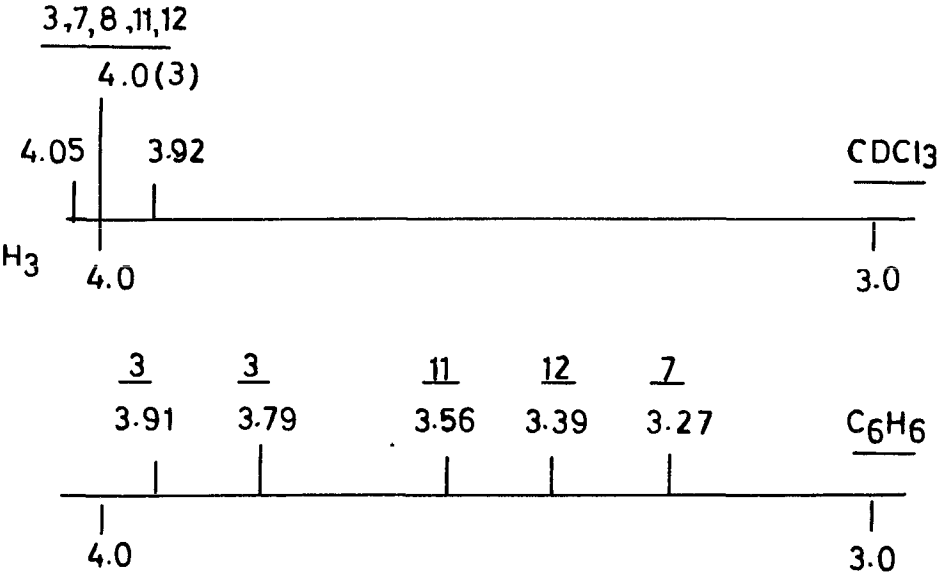
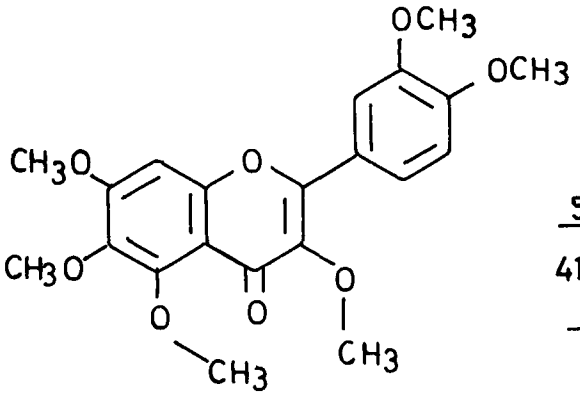
3.0

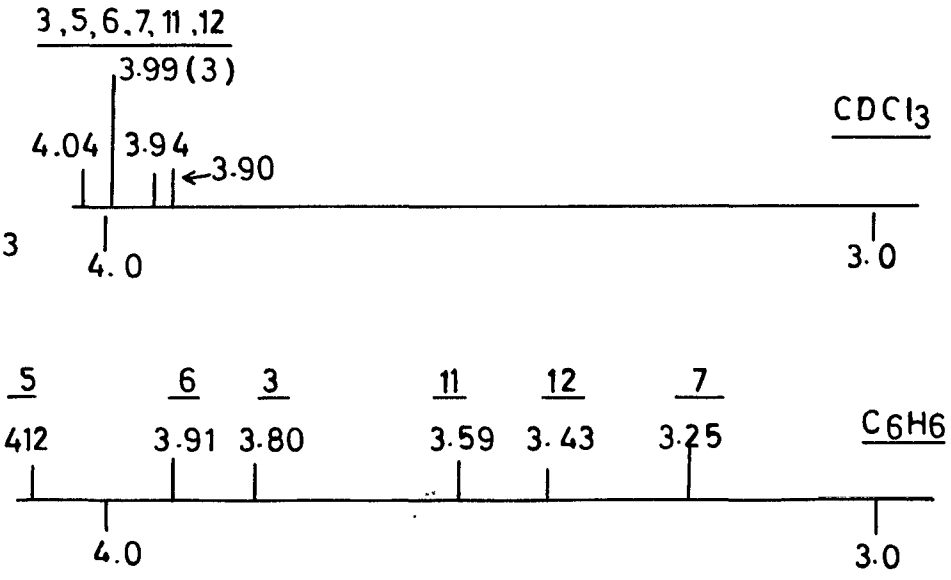

OCH3
OCH3
OCH3
CH3O
O
O
O
CH3

(XIII)


3,7,8,11,12
4.0 (3)
4.05
3.92
CDCl3
4.0
3.0
3
3
11
12
7
3.91
3.79
3.56
3.39
3.27
C6H6
4.0
3.0


OCH3
OCH3
CH3O
CH3O
O
O
O
O
CH3
CH3

(XIV)


3,5,6,7,11,12
3.99 (3)
4.04
3.94
3.90
CDCl3
4.0
3.0
5
6
3
11
12
7
412
3.91
3.80
3.59
3.43
3.25
C6H6
4.0
3.0

Methoxy groups at C-5, C-7, C-10, C-12 exhibit large positive Δ values ($\Delta = \delta\ CDCl_3 - \delta\ C_6H_6 \simeq 0.5$ to 0.8 ppm) in the absence of OMe or OH substituents ortho to these groups (Table II).

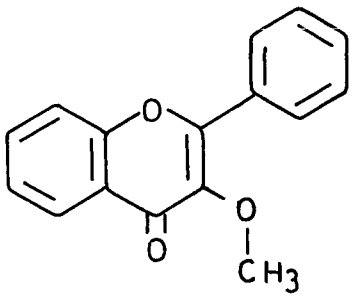
T A B L E - II

Δ Values ($\delta\ CDCl_3 - \delta\ C_6H_6$) for C-3, C-5, C-7, C-10 and C-12 methoxy resonances in the absence of ortho-substituents.

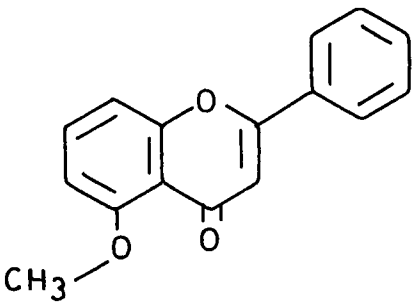
Position of OMe	Range of Δ value (ppm)
C - 3	- 0.07 to + 0.34
C - 5	+ 0.43 to + 0.58
C - 7	+ 0.53 to + 0.76
C -10	+ 0.46 to + 0.51
C -12	+ 0.54 to + 0.71

The observation is consistent with the formal ability of all these methoxy groups to conjugate with the electron withdrawing carbonyl group. This conjugation can lead to a decrease in electron density at oxygen atoms of methoxy groups in question and so enhance an association with benzene at these electron deficient sites with a resultant increased shielding effect[28-30]. The C-3 methoxy resonances are in contrast deshielded or only slightly shielded in benzene (see Table II). This observation strongly suggests

that the C-3 methoxy group in general prefers the conformation indicated in XV. In this conformation, phase independent association of benzene with the carbonyl group will have deshielding influence on the C-3 methoxy group[33-35]. Since the Δ values of the C-5 methoxy group are only slightly smaller in magnitude than those for the C-7, C-10 and C-12 methoxy groups, it is concluded that in the absence of C-6 substituent, the preferred conformation for the C-5 methoxy group is as shown in XVI (i.e. as distant as possible from the negative end of the carbonyl dipole).



(XV)



(XVI)

In contrast, the methoxy groups lacking one ortho hydrogen (i.e. flanked by two ortho methoxy groups or one ortho hydroxy and one ortho methoxy function) show small positive or negative Δ values ($\simeq$ + 0.13 to -0.12 ppm). The reason for the small positive or negatige shift is probably due to some combination of (i) steric inhibition of benzene solvation of the central methoxy group[31]

(ii) reduction in solvation of the central methoxy group (relative to anisole) due to the presence of two ortho electron donating substituents,[28-30] and (iii) solvation of the outer methoxy groups, the stereochemistry of benzene association being such as to place the central methoxy group in a region of deshielding.

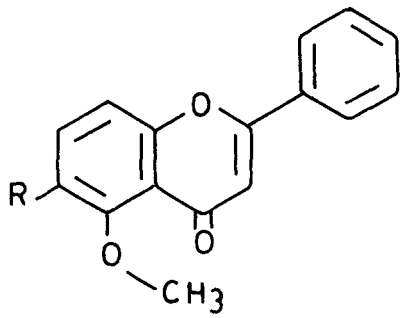
It is emphasized that the steric factors can not be the major influence, since an electron withdrawing substituent ortho to a methoxy function increases the upfield shift which is observed in benzene[30].

Since the oxygen atom attached to C-9 should have an effect similar to a hypothetical methoxy substituent at that position, it might be anticipated that in 7,8-dimethoxyflavone, the C-8 methoxy resonances would suffer only a small solvent shift. This supposition is confirmed by the data for XIII (Chart I).

In a similar manner, a methoxy group which is situated such that one neighbouring carbon atom carries a hydroxy group and the other a methoxy group, both of which can formally conjugate with carbonyl group, has a very small positive or negative solvent shift. [The C-6 methoxy of XII exhibits Δ = + 0.03 ppm (or Δ = -0.03 as an alternative assignment (Chart I)].

The solvent shift of a methoxy group at C-5 suffers

a drastic change in magnitude from a relatively large positive value to a small or negative value in the presence of a methoxy group at C-6. Such a change is in accord with expectations, since the introduction of a ortho methoxy group generally causes an algebraic decrease in Δ value (see data for XIV in chart I) and in addition a C-6 substituent should lead to a higher population of the conformer(XVII) in which the methyl of the C-5 functionality lies in close proximity to the negative end of the carbonyl dipole (which is a region of strong deshielding due to benzene association at the carbonyl group)[33-35].

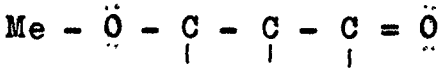


(XVII)

Benzene induced solvent shifts can be enhanced in certain cases by the addition of a little trifluoroacetic acid (TFA) to the solution of compound in benzene;[36,37] apparently protonation of certain groups enhances the benzene association at these sites. In flavones and other compounds this technique helps to distinguish between methoxy groups

which can conjugate with the carbonyl group(XVIII)and those which cannot conjugate in the ground state (XIX)[38].

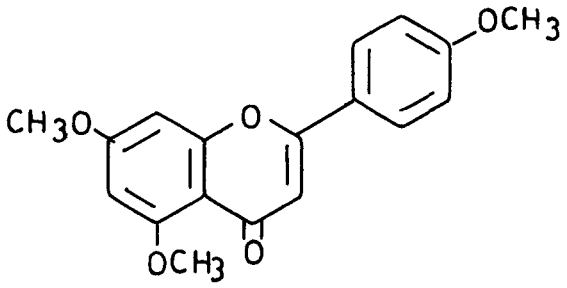
(XVII)



(XIX)

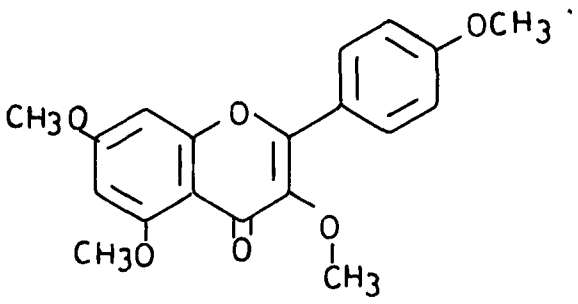
The TFA addition shifts [Δ (benzene/benzene-TFA)] for methoxy groups at C-5, C-7, C-2' and C-4' in the absence of ortho substituents are very small (Chart II). Such methoxy groups can conjugate with the carbonyl group and will thus become electron deficient; this will reduce their basicity. These result correlate with large positive benzene induced shifts observed for methoxy groups at these positions (as benzene association is always favoured at electron deficient sites).

CHART-II



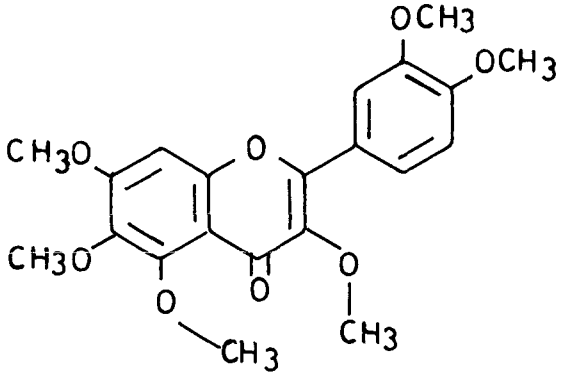
(XX)

Δ (C_6H_6/C_6H_6-TFA)(ppm)	Δ ($CHCl_3$-TFA) (ppm)	OMe group
- 0.08 to + 0.01	- 0.35	5
- 0.24 to - 0.22	-0.27 to -0.23	7
- 0.07	-0.18 to -0.14	4'



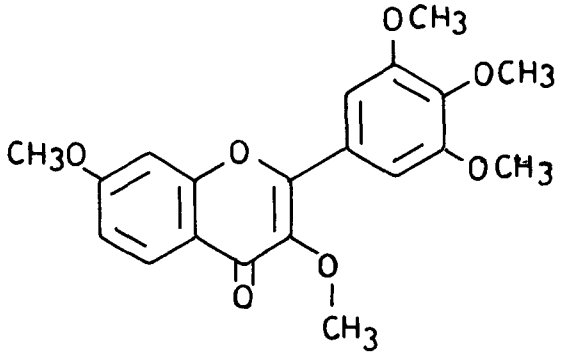
Δ (C_6H_6/C_6H_6 TFA)(ppm)	Δ ($CDCl_3$/TFA) (ppm)	OMe group
-0.05 to -0.09	-0.44 to -0.36	5
-0.22 to -0.13	-0.25 to -0.17	7
-0.03 to -0.06	-0.17 to -0.09	4'
+0.26		3

(XXI)



+0.34	-0.62 to -0.48	5
+0.29 to +0.34	-0.29 to -0.15	6
+0.18 to +0.23	-0.18 to -0.02	3
-0.23		7
+0.03 to +0.02		3'
+0.09		4'

(XXII)



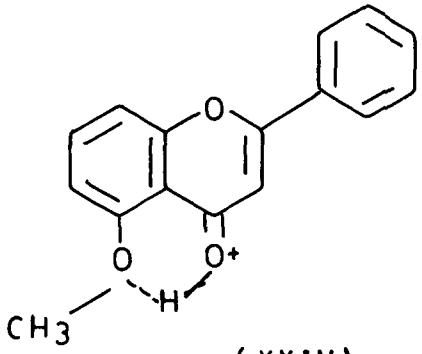
+0.43	-0.02 to +0.08	3
+0.03	-0.27 to -0.14	7
+0.21		4'
+0.11	-0.18 to -0.08	3',5'

(XXIII)

In contrast, methoxy groups at C-3 or those flanked by two ortho methoxy functions (or by one ortho methoxy and one ortho hydroxy functions) show appreciable positive

addition shifts [+ 0.18 to + 0.45 ppm (Chart II)]. It reflects an increase in the basicity of these methoxy groups as these can not overlap or conjugate with the carbonyl group in the ground state.

On change of solvent from deuterochloroform to TFA, C-5 methoxy group shows a relatively large solvent shift [Δ (δ $CDCl_3$ - δ TFA) = -0.36 to -0.44 ppm] which distinguishes it from the methoxy groups at other sites (e.g. C-7, C-4', C-2', C-6'). The latter exhibit small solvent shifts which do not permit unambiguous assignment except in very simple cases. This result is surprising, since C-5 methoxy group can conjugate with the carbonyl group, and this will tend to reduce its basicity[37]. This is shown by its large positive benzene induced solvent shift[32] and its small TFA-induced solvent shift. A possible explanation for the large TFA-induced solvent shift is the formation of a hydrogen bond between the protonated carbonyl group and the oxygen atom of the 5-methoxy group (XXIV). The carbonyl group will be protonated to a much larger extent in TFA relative to a



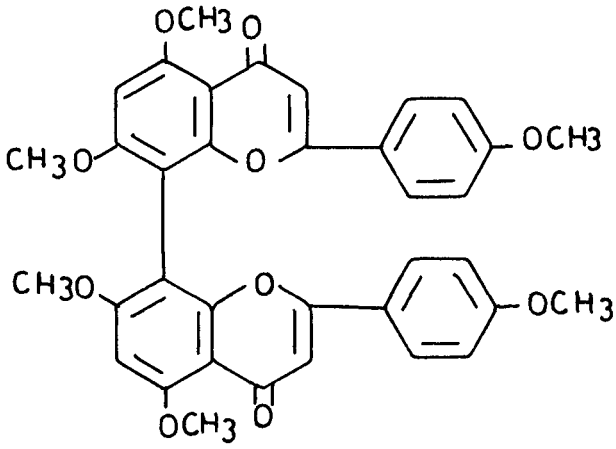
(XXIV)

solution in benzene containing only 3% TFA.

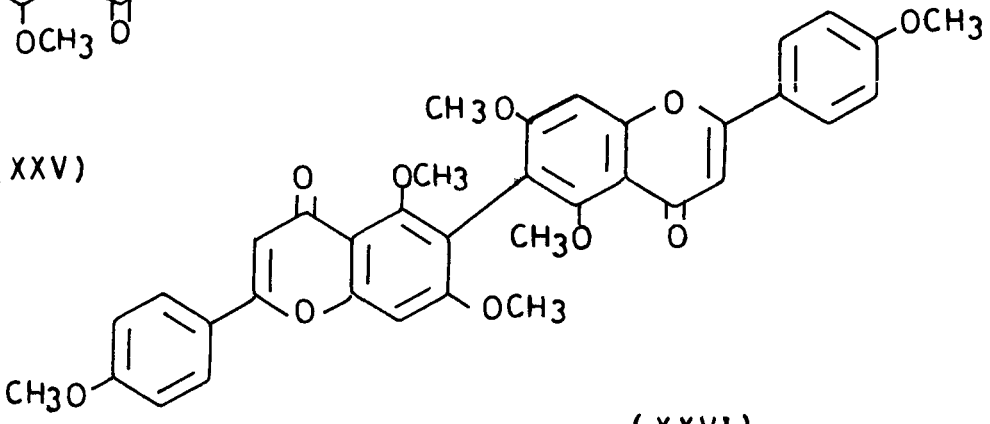
In most of the chemical investigations carried out for assigning a structure to biflavonoids, the ease of methylation has been attributed to the interflavonyl linkage being present at C-8 position in preference to C-6 position in a flavone nucleus[39]. The observation appears to be based on the steric considerations. Later studies revealed that this observation is not of general applicability and has led to erroneous assignments. This often vexing and intricate problem of distinguishing between C-6 and C-8 interflavonyl linkages in both biphenyl and biphenyl ether type biflavones has successfully been solved, in our laboratories, by using solvent induced methoxy resonances[6,27,40,41]. The following examples illustrate the application.

(a) To meet the requirements of symmetry and of PMR spectrum of cupressuflavone hexamethyl ether, structures (XXV) (8-8" linked) or (XXVI) (6-6" linked) may be postulated[40].

The assignment of 8-8" linkage was originally based on an Ullmann coupling of 8-iodo-4',5,7-trimethoxy apigenin[5]. However, in the key reaction demethylation occurred and a rearrangement to yield a 6-6" linked biflavone was not out of question.



(XXV)



(XXVI)

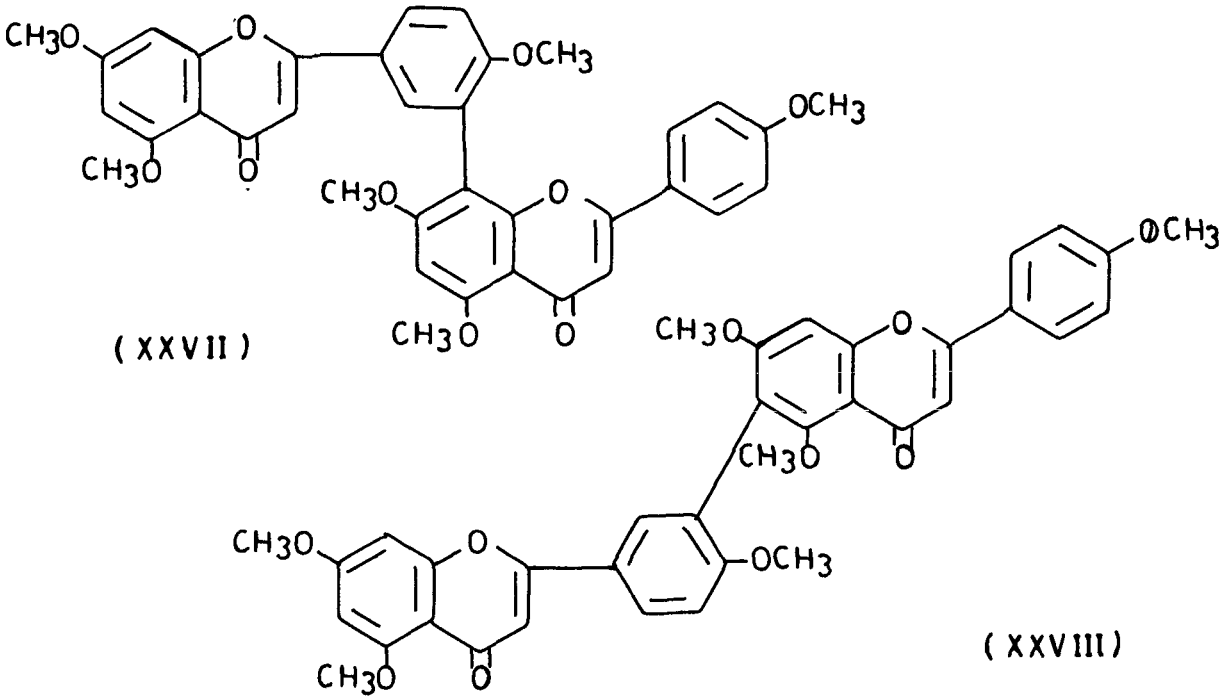
An attempt to resolve the situation was made by using solvent induced shiftsof the methoxy resonances. All the methoxy groups in structure (XXV) should shift while in (XXVI) the 5-OMe, at τ 5.88, should not move. In fact solvent change from $CDCl_3$ to C_6H_6 caused the following shifts.

5 - OMe	53 c/s
7 - OMe	75 c/s
4'- OMe	49 c/s

This clearly established that cupressuflavone was 8-8" linked and not 6-6" linked biflavone[40].

(b) In amentoflavone hexamethyl ether all the methoxy groups on the change of solvent from $CDCl_3$ to C_6H_6 moved

upfield as with cupressuflavone hexamethyl ether, showing that each methoxy group had at least one ortho proton, thus establishing a 3'-8" (XXVII) rather than 3'-6" linked structure (XXVIII) in the series[41].

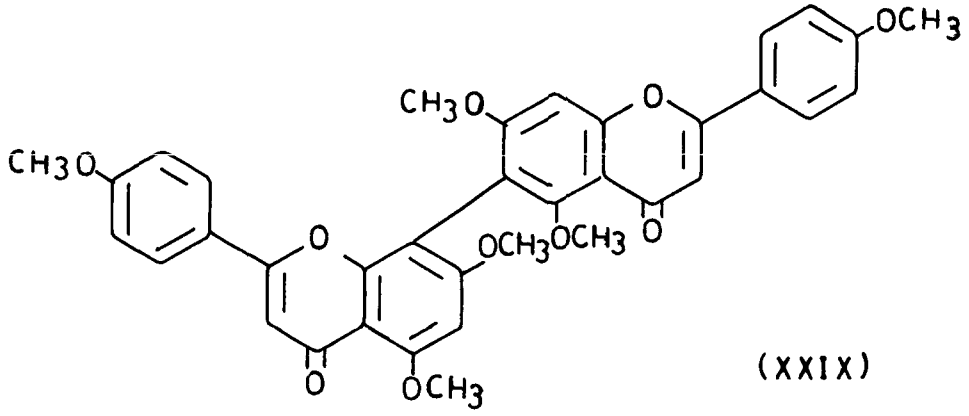


(XXVII)

(XXVIII)

(c) NMR spectrum of agathisflavone hexamethyl ether[6] showed the compound to be unsymmetrical. The presence of two sets of A_2 B_2 protons further indicated that neither ring B nor ring E was involved in interflavonyl linkage between two monoflavonoid units. The absence of any meta coupled pair of protons associated with ring A or D ruled out the presence of linkage at C-3 or C-3". This left ring A and D implicated in the linkage and as the compound

was unsymmetrical i.e. neither 8-8" nor 6-6" linked, the linkage must be 6-8" (XXIX).

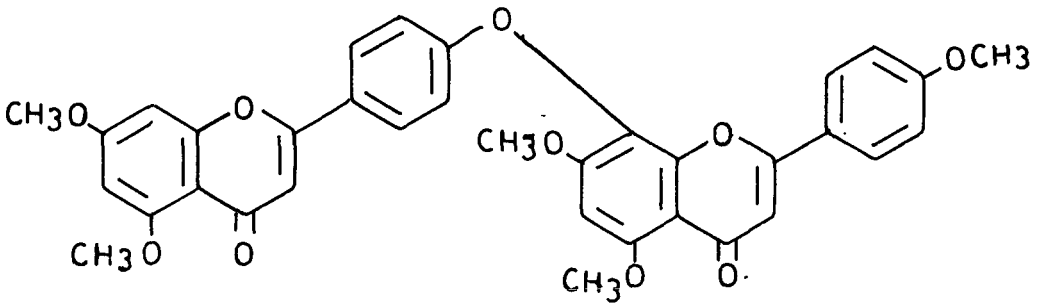


(XXIX)

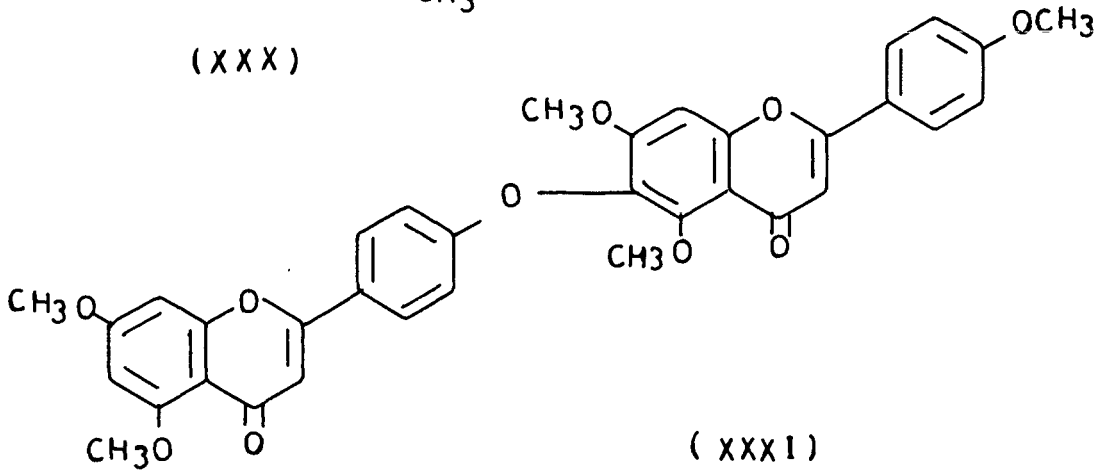
In agathisflavone hexamethyl ether on change of solvent from deuterochloroform to benzene five methoxy groups (each with an ortho hydrogen atom) behaved as expected and showed large upfield shifts. One methoxy group was unique in that upto ~ 50% dilution with benzene no shift was seen and then a strong downfield shift was evidenced, a phenomenon seen neither in amentoflavone nor in cupressuflavone hexamethyl ethers. It was reasonable to assume that the methyl group in question was the one at C-5, flanked by ring D on one side and a carbonyl group on the other. This result supported the structure (XXIX) for agathisflavone hexamethyl ether.

(d) Despite the large amount of work that had been devoted to the elucidation of the structure of hinokiflavone and its

derivatives, the structure of only one sample was fully settled. The elegant total synthesis of hinokiflavone pentamethyl ether by Nakazawa[42] proved that the sample produced by Kawano[43] had the 4'-O-6" linkage (XXXI). Previously the compound had been assigned the 4'-O-8" linkage (XXX) on the basis of spectral and degradative evidences[44,45].



(XXX)



(XXXI)

A comparative study[46] of the positions of methoxy groups in PMR spectrum of hinokiflavone pentamethyl ether as compared with those of the corresponding monomeric flavone methyl ethers also indicated structure (XXX).

Under demethylating conditions (XXX) was converted to (XXXI)[42] and this meant that synthesis of these compounds

in which at any stage demethylation occurred, could not be looked upon as unambiguous. Seshadri and co-workers[47] further recorded that they were unable to distinguish the two isomers by standard chemical means and although the PMR spectra differ in details (Table III) it is difficult to use these diagnostically.

T A B L E - III*

Proton	XXXI	XXX
2"', 6"'	2.20 (d) J = 9	2.56 d J = 9
3"', 5"'	3.06 (d) ,,	3.19 d ,,
2', 6'	2.12 (d) ,,	2.16 d ,,
3', 5'	2.98 (d) ,,	2.94 d ,,
3', 3"	3.38, 3.41	3.41, 3.44
6"	-	3.49
8"	2.95	-
6.8	⌈3.45,3.63 J = 2 c/s⌋	⌈3.51,3.64 J=2 c/s⌋
OMe	6.06-6.09	5.95, 6.02, 6.05, 6.10, 6.21

d = doublet

*Spectra were run at 100 M c/s (TMS as an internal standard = τ 10.00). Values of J in c/s.

On change of solvent from $CDCl_3$ to C_6H_6 (XXX) showed methoxy shifts precisely as expected from a compound with five methoxy groups unhindered to solvation by benzene

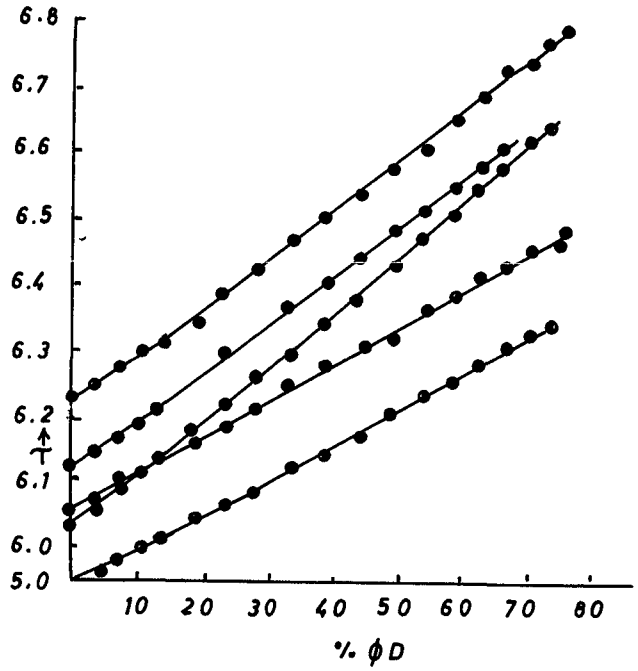

6.8
6.7
6.6
6.5
6.4
6.3
6.2
T
6.1
6.0
5.0
10 20 30 40 50 60 70 80
% ϕD

Fig. I

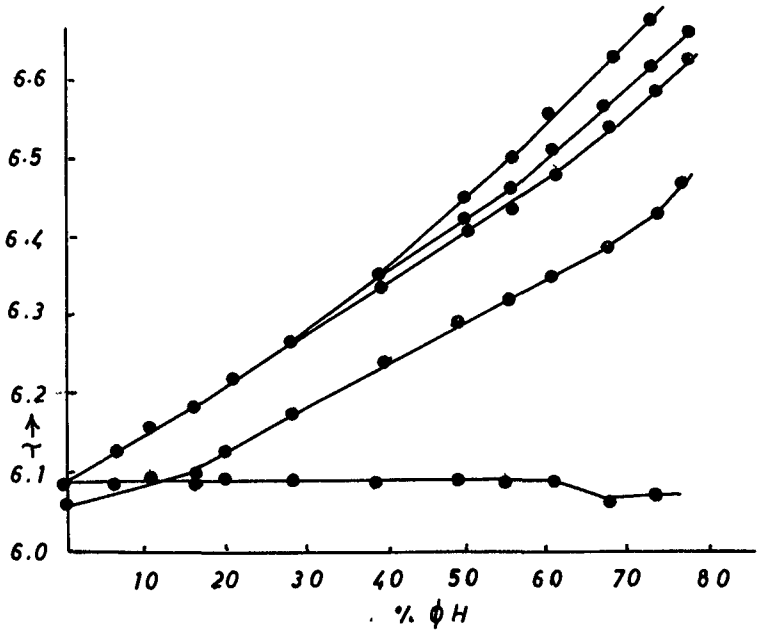

6.6
6.5
6.4
6.3
6.2
T
6.1
6.0
10 20 30 40 50 60 70 80
% ϕH

Fig. II

(Fig. I). On the other hand isomer (XXXI) showed methoxy shifts as 34 c/s, 54 c/s, 57 c/s, 64 c/s and 1 c/s (Fig. II).

These shifts are well within the range for four unhindered methoxy groups and one hindered methoxy group.

This difference between Fig. I and Fig. II is clear and seems to meet the requirements for a simple test to distinguish between 6-linked and 8-linked compounds[27].

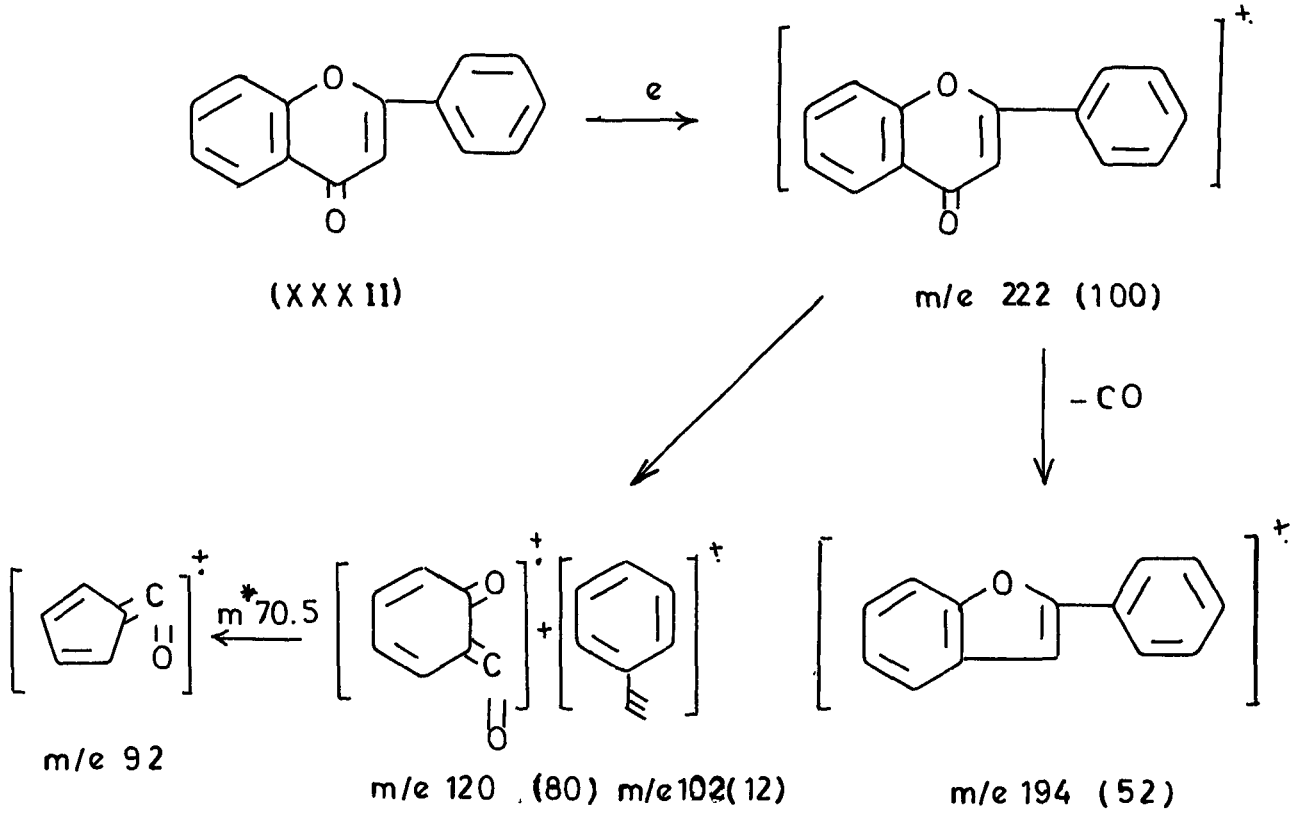
MASS SPECTROMETRY

The mass spectra of a wide variety of organic compounds have been studied only during the last few years. The main reasons underlying the sudden burst of activity in the field have been the introduction of inlet systems suitable for volatilization of relatively high molecular weight (M^+ 300-1200) organic materials and the realization that fragmentation pattern in many cases be simply related to the structure of the intact molecule. Recently a number of papers on the evaluation of structure-fragmentation pattern relationships in mono- and biflavonoids have appeared.

FLAVONES

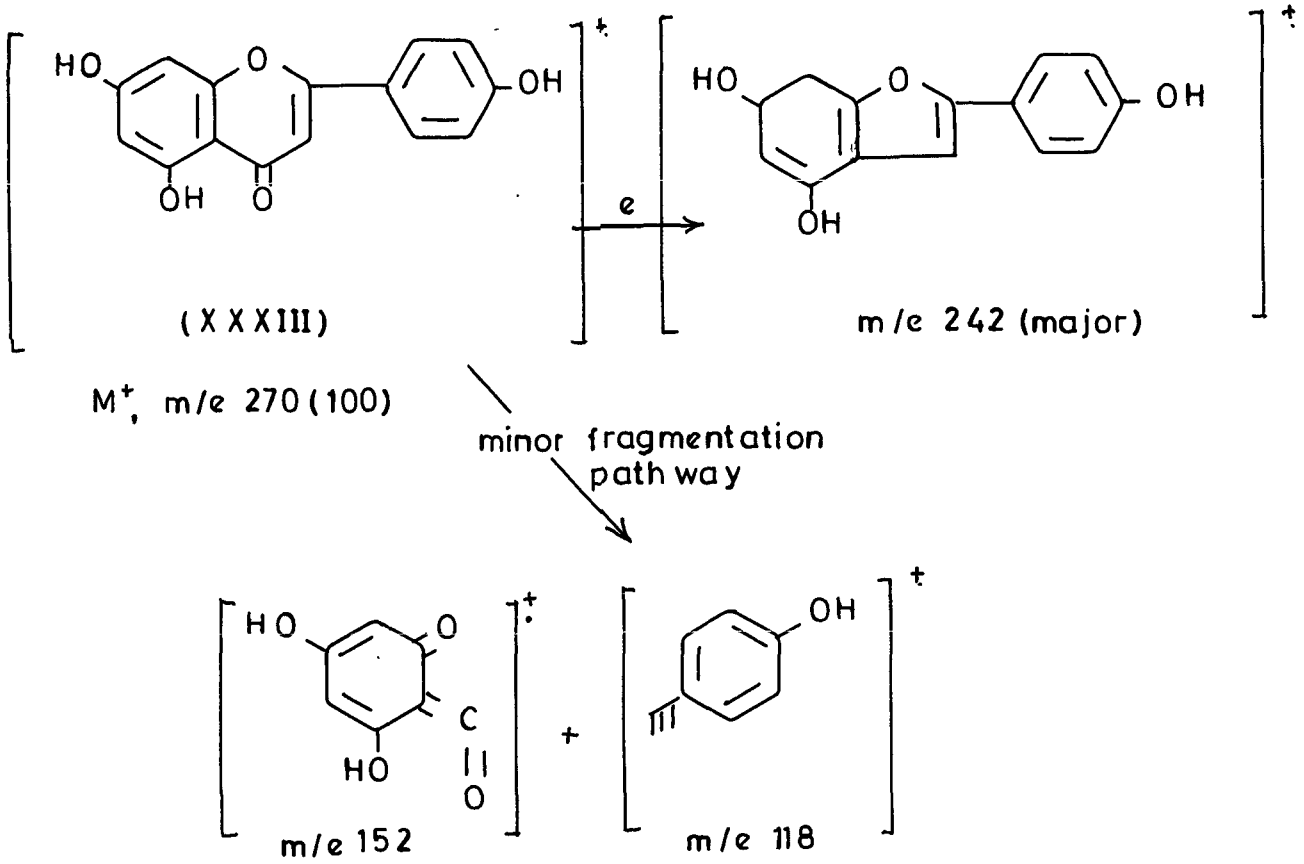
Flavone (XXXII)[48] itself gives the molecular ion as the base peak at m/e 222 (100) and shows subsequent loss of

one hydrogen to give an ion, m/e 221(33) of doubtful structure, while one oxygen is eliminated as CO [m/e 194 (52)]. But more readily the flavone, by fission of the heterocyclic ring, gives two ions, one with a quinonoid, [m/e 120 (80)] and the other a phenyl-acetylene [m/e 102 (12)] structures. The ion, m/e 92 arises by loss of CO from the ion at m/e 120 (meta stable peak at 70.5).



Apigenin (XXXIII)[49] has the parent molecular ion as the base peak and an abundant fragment ion m/e 242 corresponding to the loss of carbon monoxide. Fragment ions of much less abundance correspond to RDA (Retero Diels-Alder)

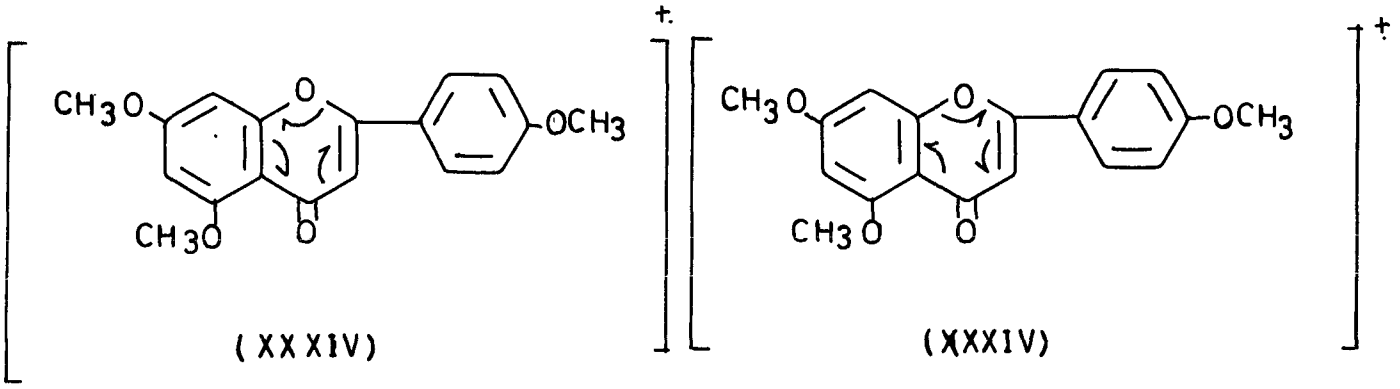
fission in the heterocyclic ring.



The discrepancy between the results obtained lies in the importance assumed by breakdown via RDA reaction in the natural products as compared with the parent flavone. In the highly oxygenated natural products this fragmentation is minor (15-16% of molecular ion) whilst in flavone itself the peak due to species with a quinonoid structure is 80% of the intensity of the molecular ion. It appears, therefore, that oxygenation of nucleus profoundly influences the breakdown observed. Presumably, if the initially produced ion radical can be stabilized by mesomerism over a number of

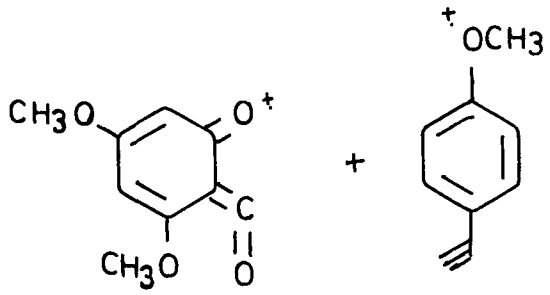
oxygen atoms, then breakdown via RDA is strongly diminished. These minor breakdowns may still prove to be of diagnostic value as they frequently represent the only even numbered peaks in their particular region and hence are readily distinguished.

The fragmentation pattern of apigenin trimethyl ether (XXXIV)[50] may be represented as follows:



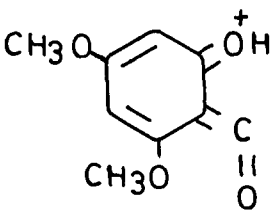
M^+, m/e 312

Route I



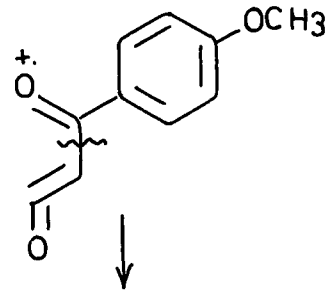
m/e 180

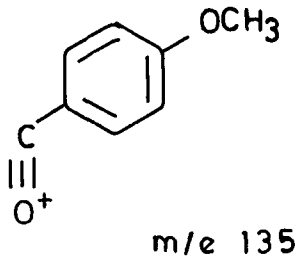
m/e 132



m/e 181

Route 2

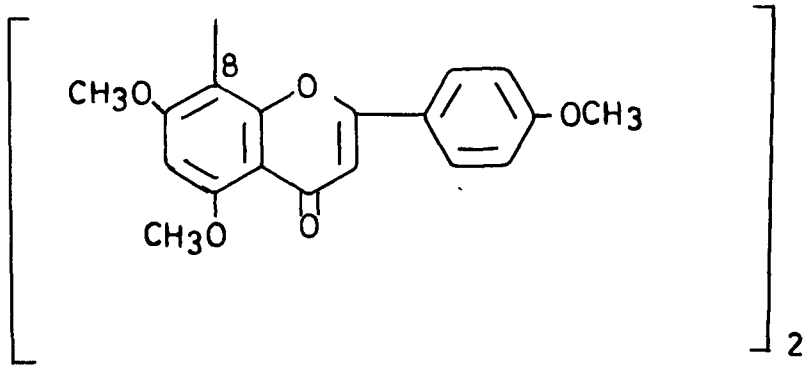




m/e 135

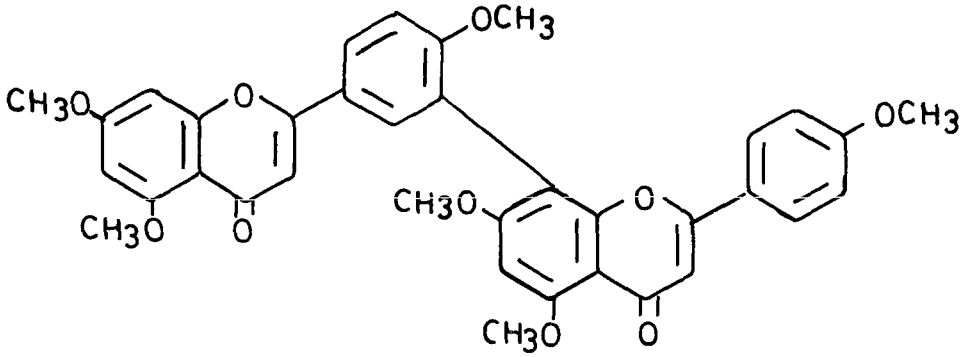
Seshadri et al[50] have reported that the fragmentation patterns of biphenyl type biflavones viz cupressuflavone hexamethyl ether and amentoflavone hexamethyl ether are similar, molecular ion being the base peak in each case. There are differences in the intensities of the corresponding ions in their spectra, chiefly due to structural variations. Steric factors also seem to play an important role in influencing the breakdown mode and internal condensations. These factors become so much dominant in agathisflavone hexamethyl ether[51] that the ion at m/e 311 appears as base peak instead of molecular ion m/e 622 (90). Main peaks which appear in their mass spectra are given below:

Cupressuflavone hexamethyl ether (XXV):



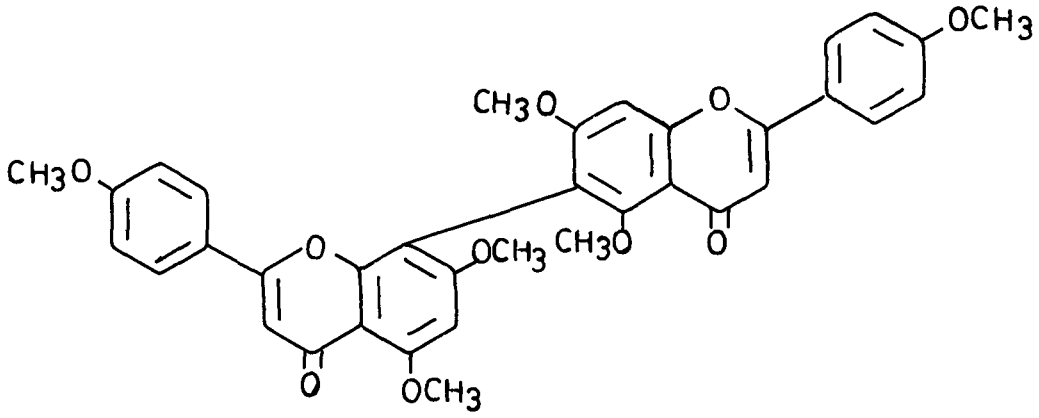
Main peaks appear at 622 (100); 621 (38); 607 (8); 592 (18); 576 (4); 312 (7); 311 (14); 245 (11); 135 (26) and 132 (14).

Amentoflavone hexamethyl ether (XXVII):



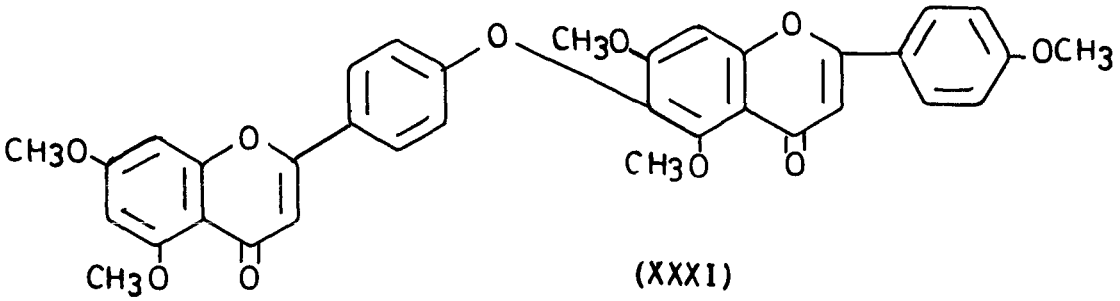
Main peaks appear at 622 (100); 621 (31); 607 (33); 592 (8); 576 (10); 312 (2); 311 (5); 245 (5); 135 (16); and 132 (8).

Agathisflavone hexamethyl ether (XXIX):



Main peaks appear at 622 (90); 607 (54); 591 (98); 573 (24); 561 (15); 521 (12); 497 (24); 325 (20); 311 (100); 281 (12); 245 (22) and 135 (65).

The mode of fragmentation of hinokiflavone pentamethyl ether (XXXI)[50] which contains a biphenyl ether system, is considerably different from those of amentoflavone, cupressuflavone and agathisflavone hexamethyl ethers. The base peak in this case appears at m/e 313 and the molecular ion amounts to 39% of this peak. This may be attributed to the presence of easily rupturable biphenyl ether linkage.

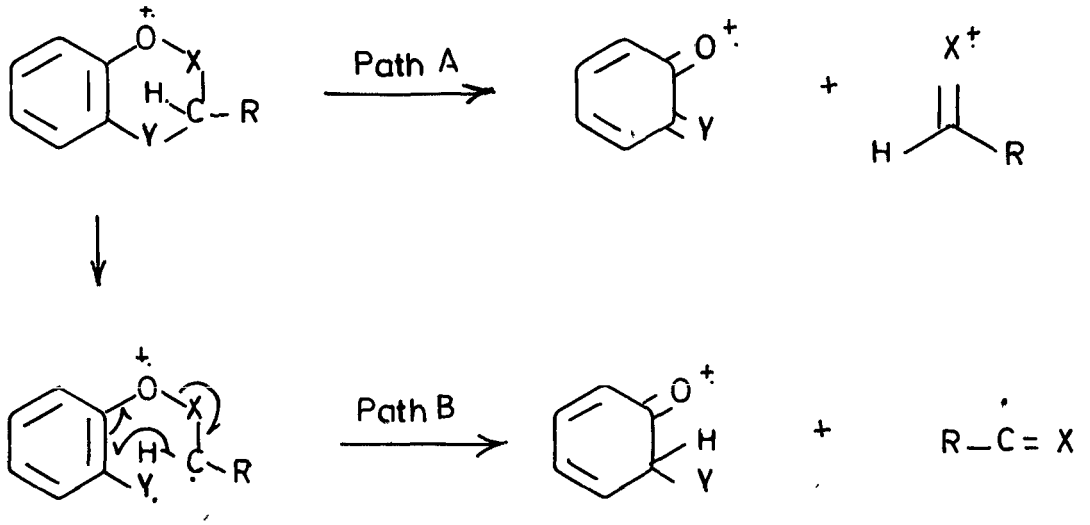


(XXXI)

Main peaks appear at 608 (39); 607 (12); 593 (36); 579 (11); 431 (7); 327 (23); 313 (100); 312 (22); 311 (22); 297 (29); 296 (75); 281 (22); 181 (11); 135 (19) and 132 (18).

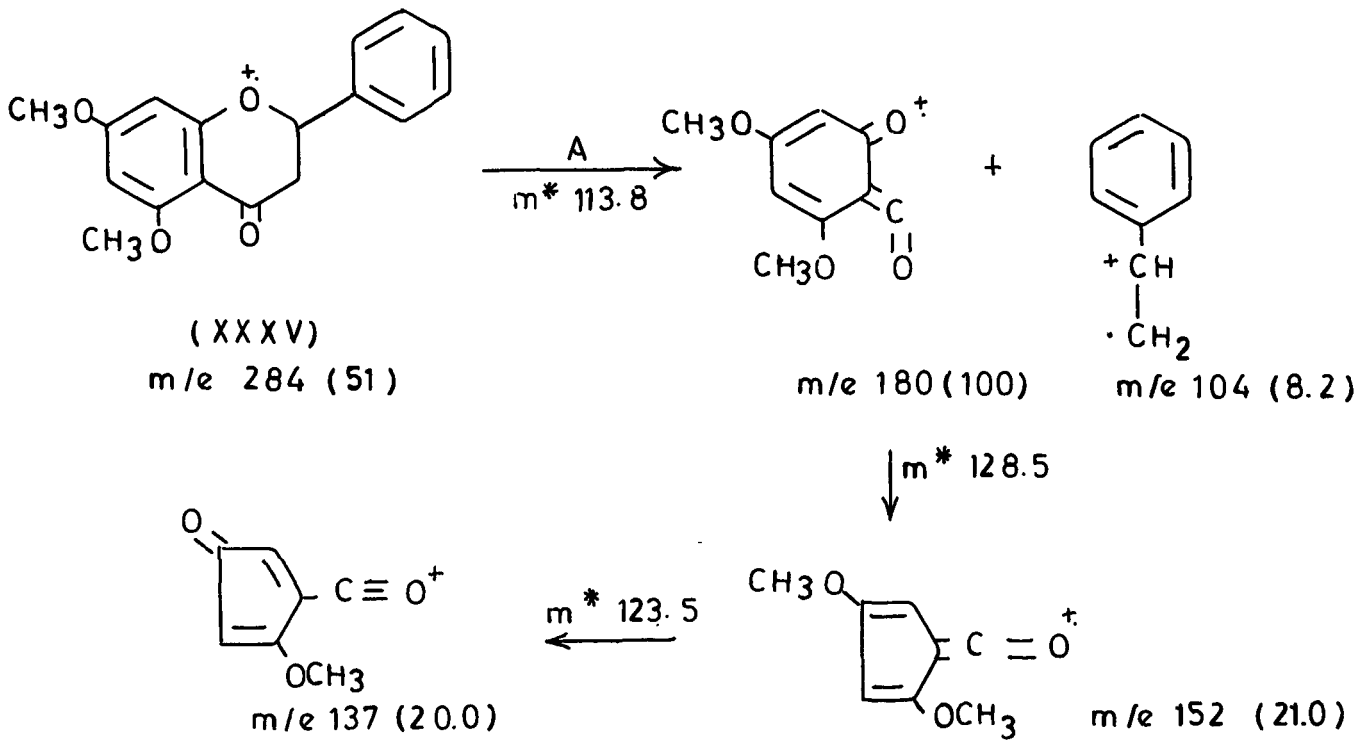
FLAVANONES

In case of reduced flavonoids, in which the heterocyclic ring is no longer aromatic, breakdowns by paths A and B are of great importance as they lead to clean cut, characteristic spectra[52].



Mass spectrum of 5,7-dimethoxy flavanone (XXXV)[52] may be rationalized as follows:

The major pathway involves breakdown by mode A to give the fragments of m/e 180 and m/e 104, the former containing two methoxy groups taking most of the charge. This species loses carbon monoxide to give fragment at m/e 152, the series being terminated by the loss of a methyl radical to give the even electron species at m/e 137.



(XXXV)
m/e 284 (51)

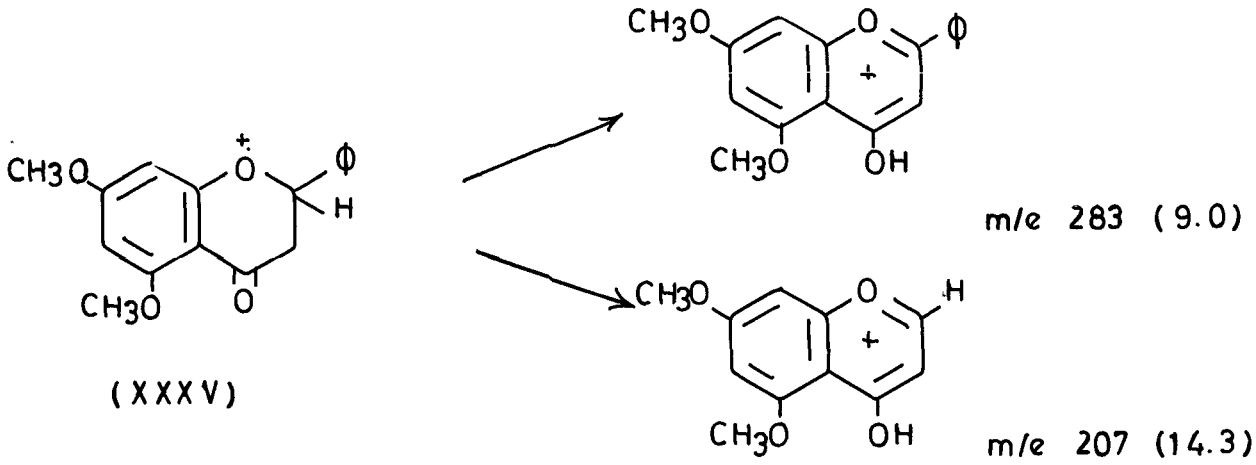
m/e 180 (100)

m/e 104 (8.2)

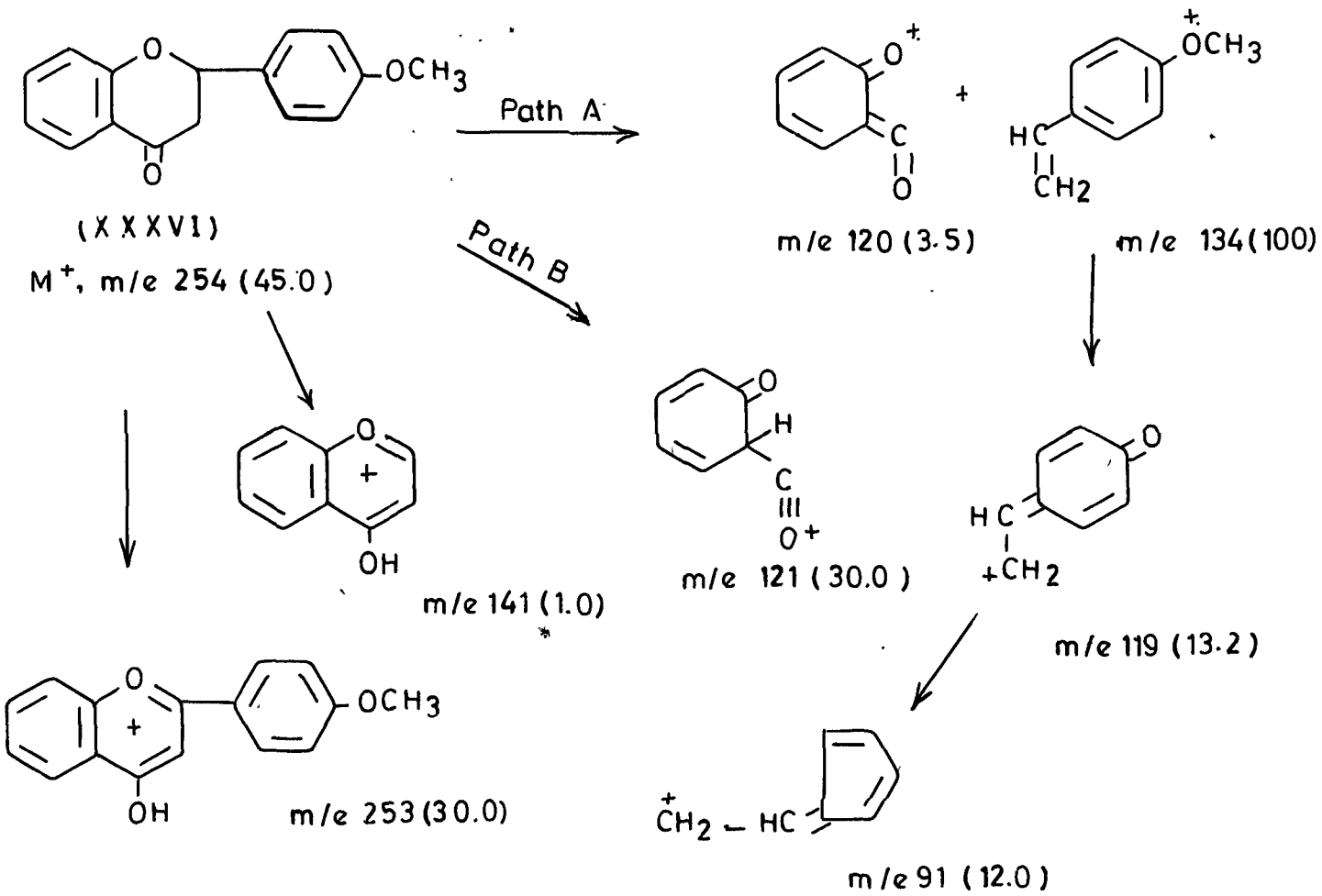
m/e 137 (20.0)

m/e 152 (21.0)

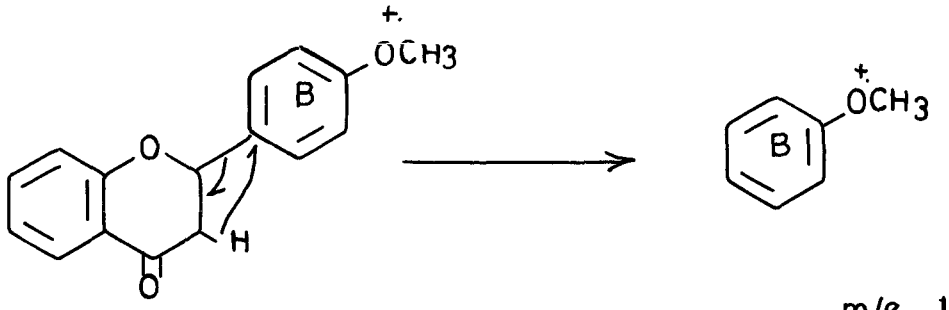
Another method of breakdown, that helps to characterise the flavanones is the loss of either a hydrogen atom or an aryl radical from the molecular ion to give even electron fragments.



A very similar breakdown pattern is found for 4'= methoxy flavanone (XXXVI)[52], once more the fragment with

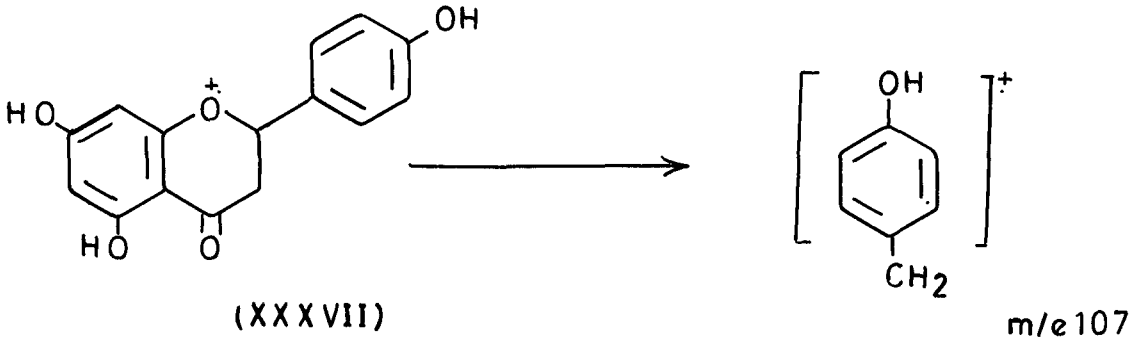


methoxyl group taking nearly all the charge. Path B is more noticeable, the breakdown scheme being shown on page 41. A further peak is at m/e 108 arising from a hydrogen transfer reaction.



(XXXVI) m/e 108 (10.0)

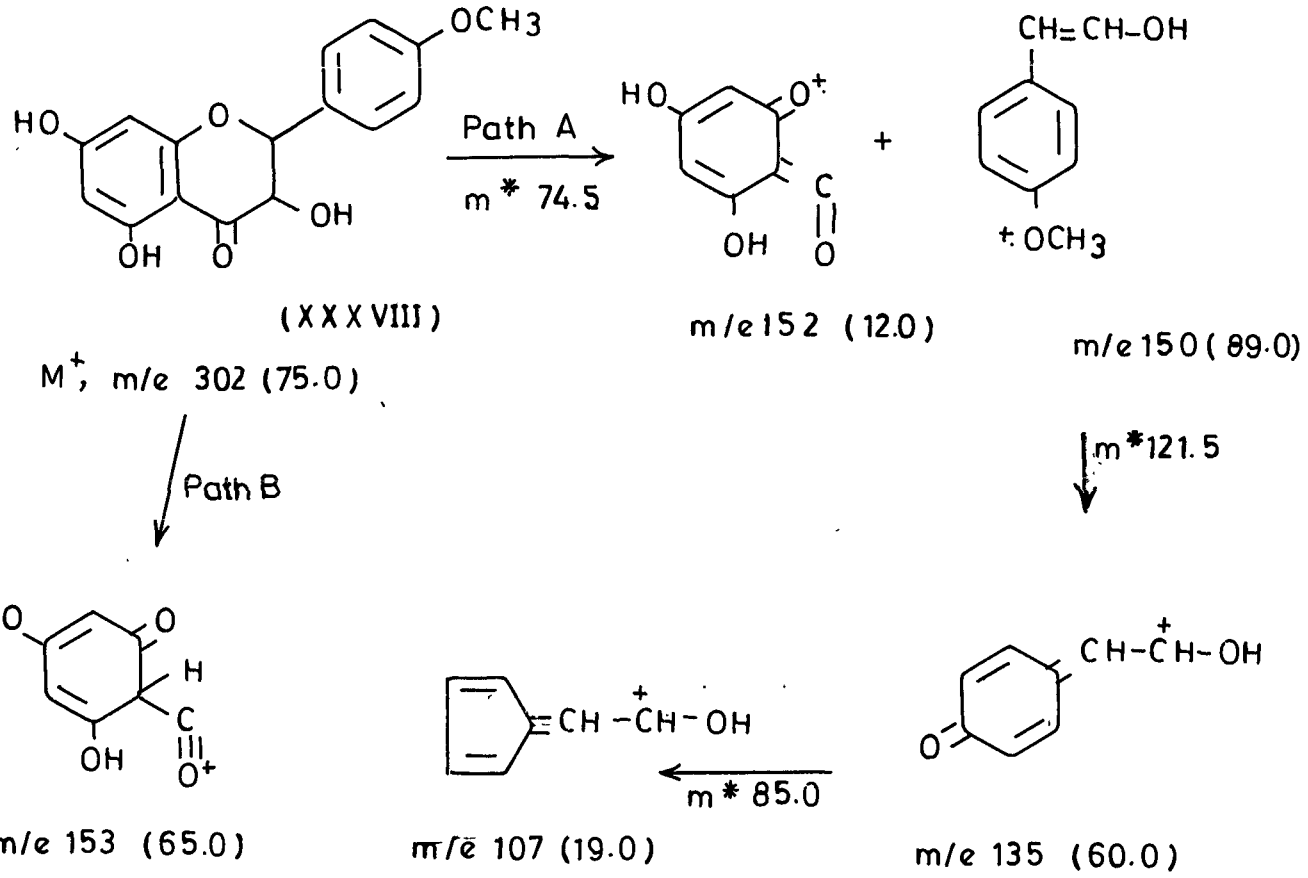
The presence of a hydroxyl/methoxyl group at C-4 position of ring B facilitates, by enhanced resonance stabilization of the resulting fragment ion, the formation of p-hydroxy benzyl/p-methoxy benzyl fragment (or its tropolium ion). p-Hydroxy/p-Methoxy benzyl ion appears as peak of significant intensity in the mass spectrum of naringenin/its trimethyl ether (XXXVII)[37].



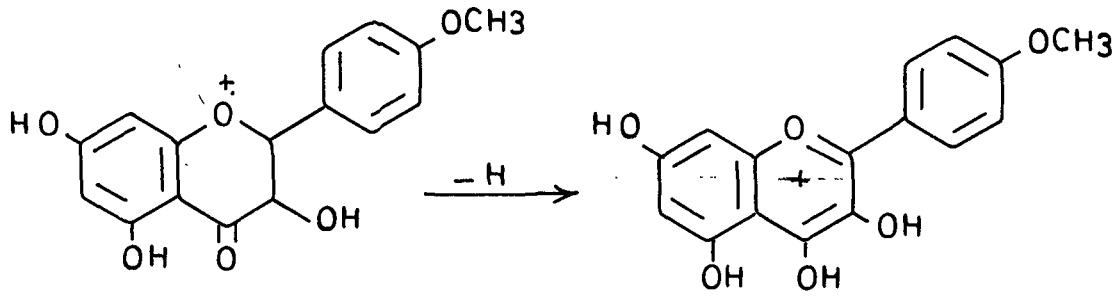
(XXXVII) m/e 107

The spectrum of 3,5,7-trihydroxy-4'-methoxy flavanone (XXXVIII)[52] is of great interest as it was the first reduced flavonoid encountered in which the base peak is neither the

molecular ion nor a fragment arising from breakdown via path A. However, this type of breakdown as well as path B, are found as shown below:

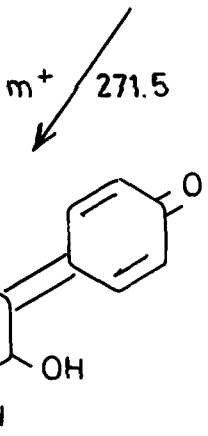


The loss of a hydrogen atom followed by the loss of a methyl radical is of importance, but the base peak is found at m/e 137. The metastable peak at m/e 62.2 indicates that this fragment is formed directly from the molecular ion. Several processes can give rise to this species. as shown on the next page.

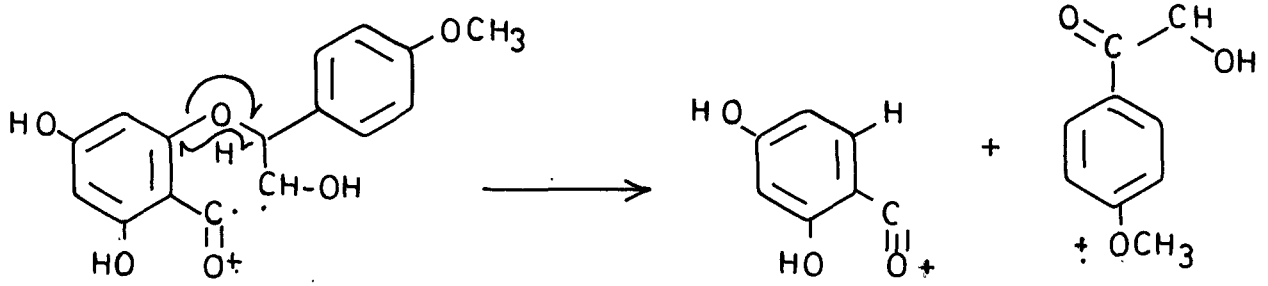


m/e 301 (26.0)

(X X X VIII)

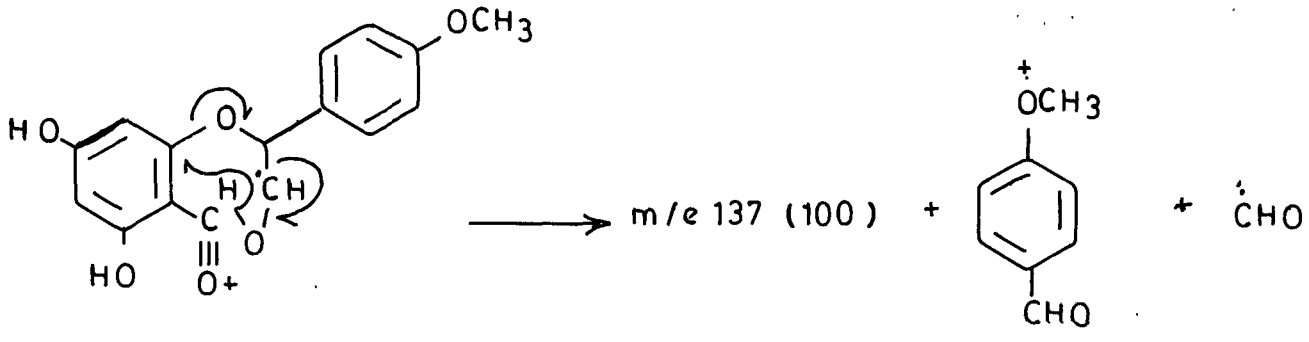


m/e 286 (9.5)



(XXX VIII)

m/e 137 (100.0) m/e165 (1.1)

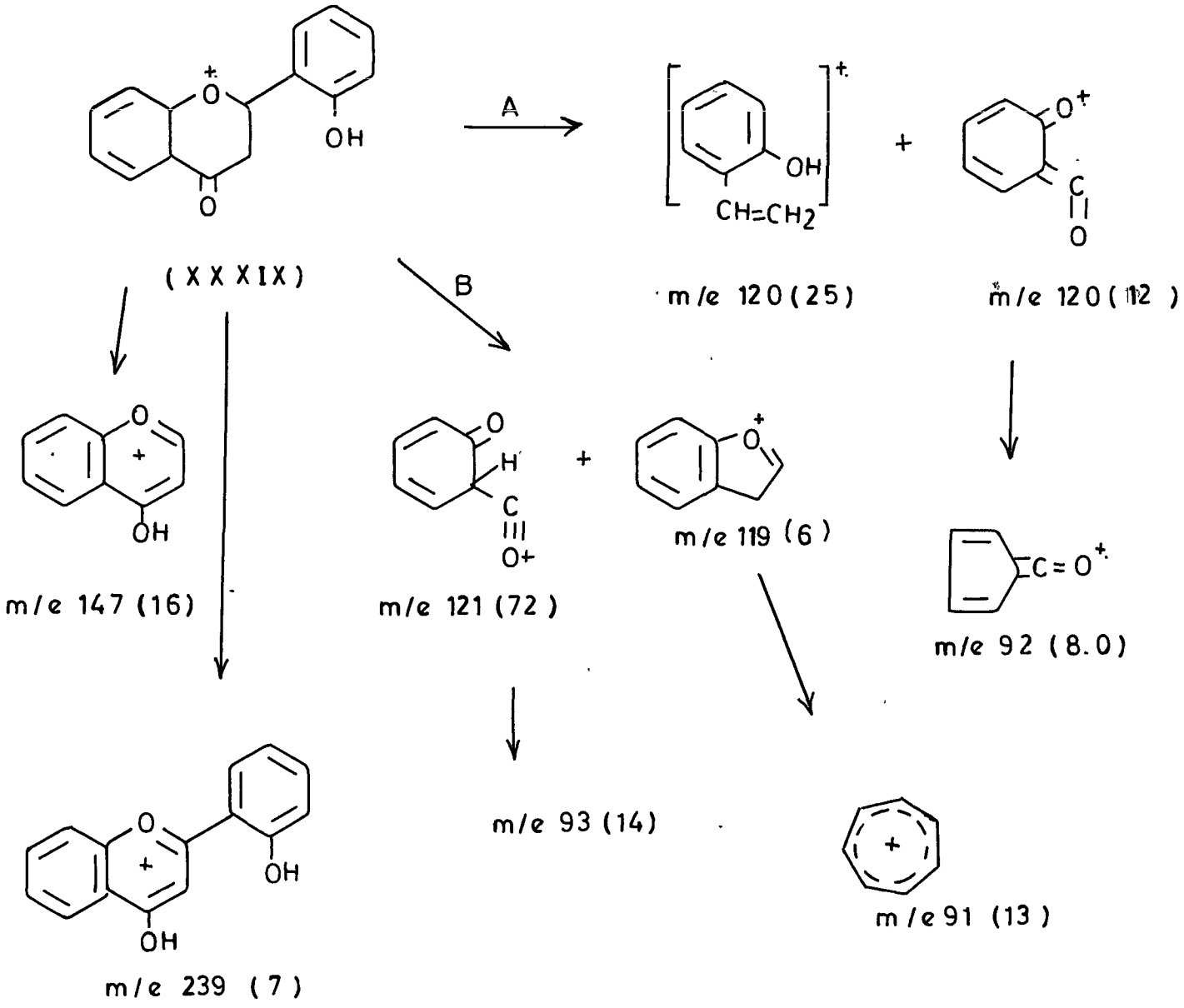


(XXXVIII)

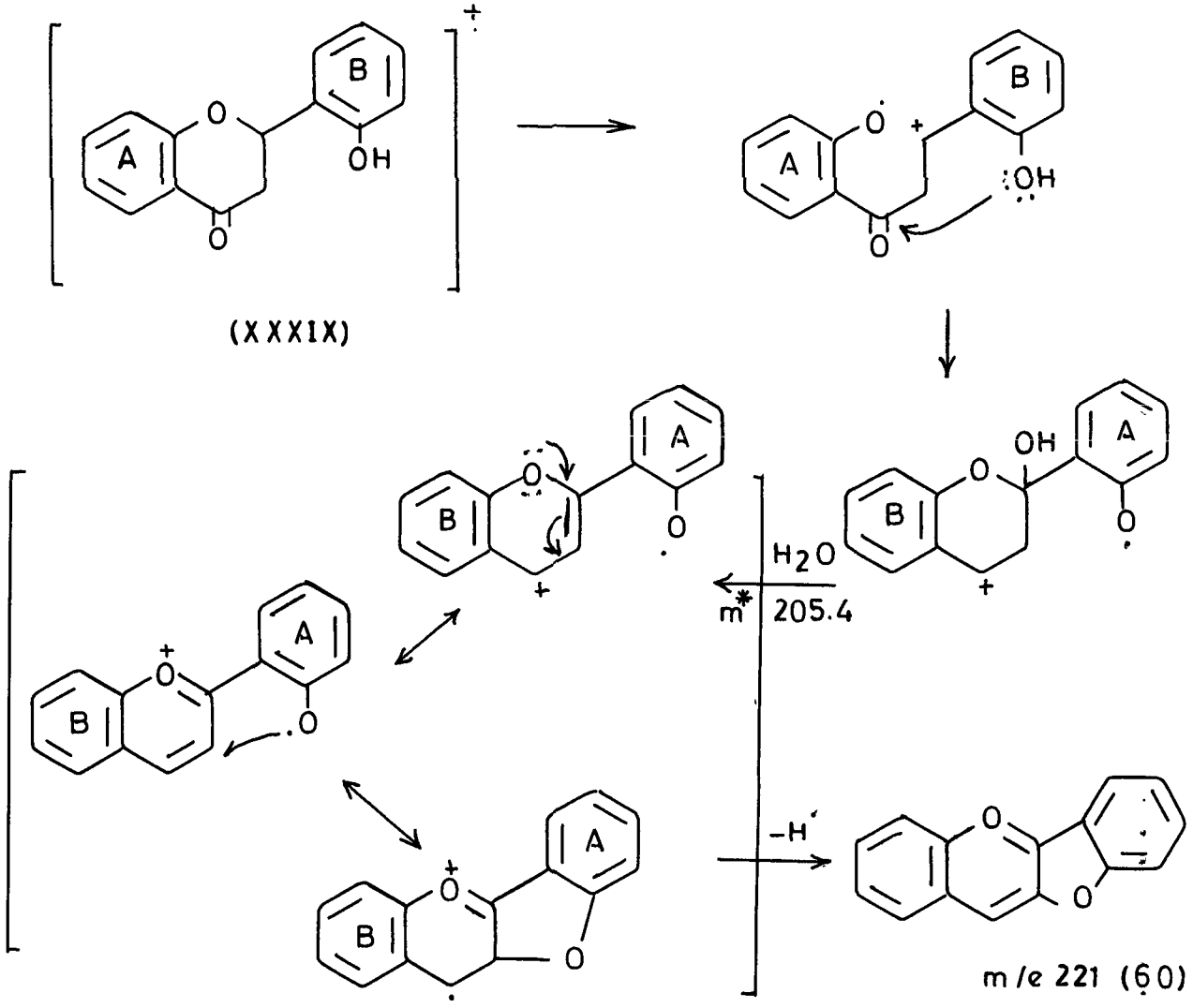
m/e 136 (8.7)

In the case of 2'-hydroxy flavonoids strong intramolecular interactions occur and the breakdown pattern becomes so profoundly modified that it is frequently difficult to classify the substance by reference to standard breakdown patterns[53].

2'-Hydroxy flavanone (XXXIX)[53] showed breakdown patterns A and B as well as the loss of phenyl or hydrogen radical from C-2 to give even electron species, but the



base peak was at M-18 and the third largest peak at M-19. It has been proposed that these peaks arise by ring opening of the molecular ion followed by ring closure on to the 2'-hydroxy group as shown below:

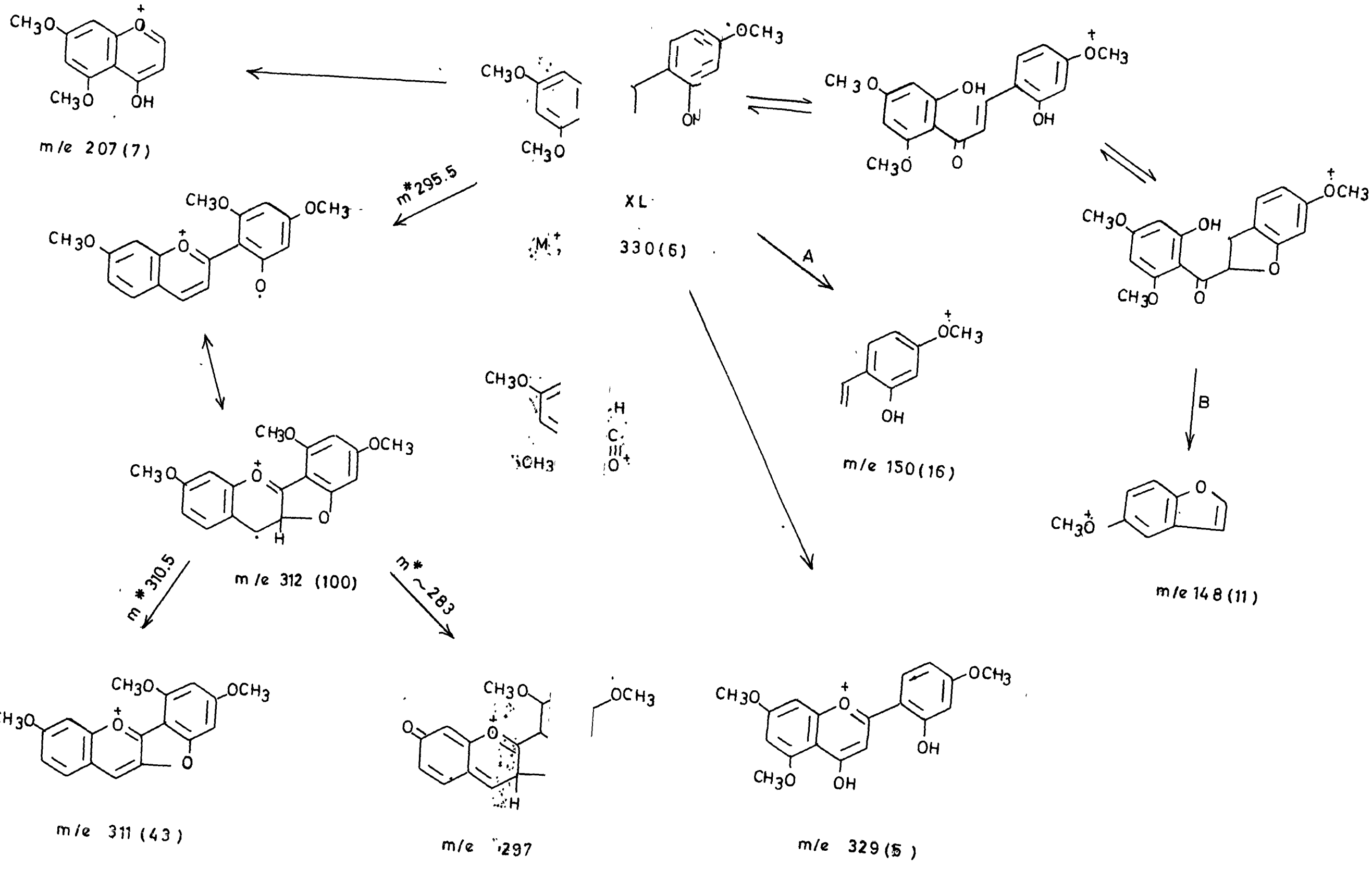


(XXXIX)

m/e 222 (100)

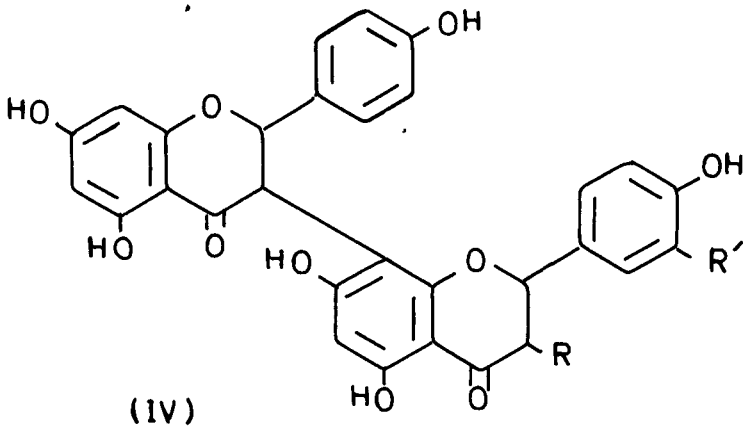
Another example of this flavanone cleavage is shown by 2'-hydroxy-4'5, 7-trimethoxy flavanone (XL)[53]. Once more the base peak is produced by loss of water, whilst further loss of a proton yields the third largest peak. Further,

SCI ME-I


m/e 207 (7)
m*295.5
XL
M+
330 (6)
A
B
m/e 150 (16)
m/e 148 (11)
m*310.5
m/e 312 (100)
m*~283
m/e 311 (43)
m/e 297
m/e 329 (5)

loss of a methyl group in this heavily substituted flavanone is possible to yield p-quinonoid structure ion at m/e 297. Cleavage by route B gives the abundant ion at m/e 181, though all ions derived by routes A and B can be seen. Of interest is the ion of mass 148, represented as 6-methoxy benzofuran. This suggests that ring closure has occurred on to a chalcone form followed by cleavage. This is in agreement with the finding that chalcones are equivalent to flavanones in mass spectrometer. The breakdown may be summarized as in scheme I.

The mass spectrometry has been used by Jackson et al[7,54] to elucidate the structures of biflavonoids of GB series containing two flavanone units linked through 3-8".



(IV)

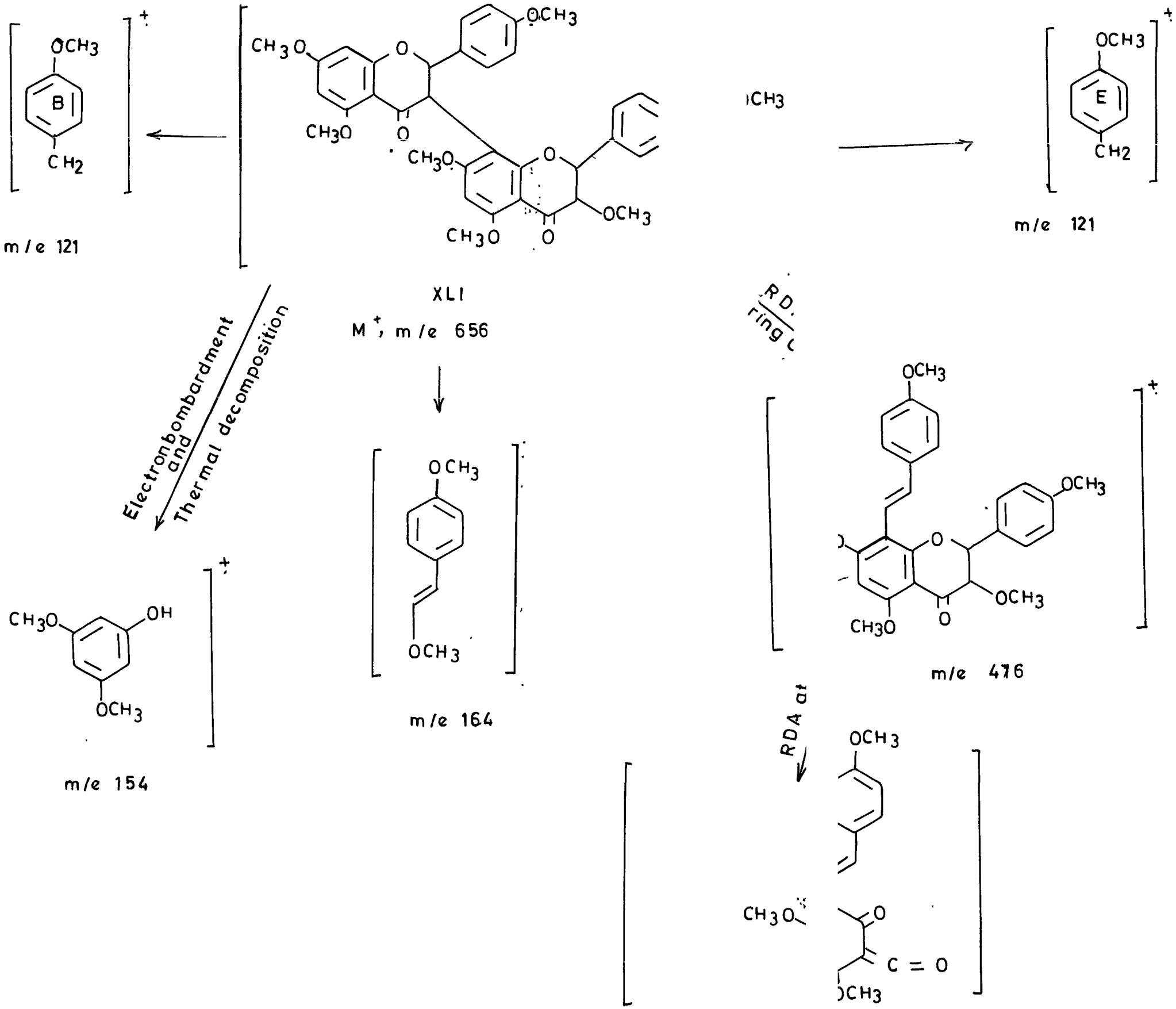
		R	R'
(a)	GB-1	OH	H
(b)	GB-1a	H	H
(c)	GB-2	H	OH
(d)	GB-2a	OH	OH

The mass spectrum of GB-1 heptamethyl ether (XLI) (M^+, m/e 656) showed the presence of ions at m/e 121, 154, 181, 312 and 476. The presence of ions at m/e 154 and 181 consistent with the fragments $[C_6H_3(OMe)_2OH]^{+\cdot}$ and $[C_6H_2(OMe)_2OH\ CO]^{+\cdot}$ supported the presence of phloroglucinol ring system derived from 5,7-dihydroxy flavanone. In addition the presence of another aromatic ring was suggested by an ion at m/e 121 consistent with a $[MeO.C_6H_4.CH_2]^{+\cdot}$ fragment. Mass spectrum also supported the nature of the linkage since the fragmentation of molecular ion at m/e 656 can be rationalized by RDA reaction of flavanones first at ring C to give a fragment ion at m/e 476 followed by a similar fragmentation at ring F to give an ion at m/e 312. This two stage breakdown fragmentation pattern is fully substantiated by the presence of metastable peaks[54]. These results can only be accommodated by a linkage from the oxygen heterocyclic ring C to the phloroglucinol ring D (Scheme II).

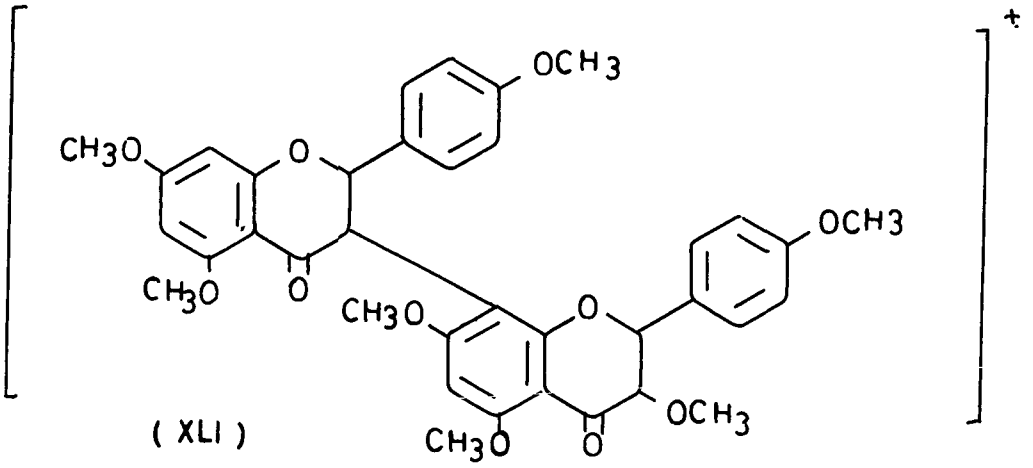
Pelter[8] suggested that the production of fragment ion at m/e 312 can be explained through another mode of fragmentation (Scheme III) while the formation of most of other ions is explainable by RDA reaction characteristic of flavanones.

The production of dimethyl phloroglucinol ion at m/e 154 seems quite odd as simple analogous flavanones do not show this ion. On the basis of previous findings,

S C H E M E — II

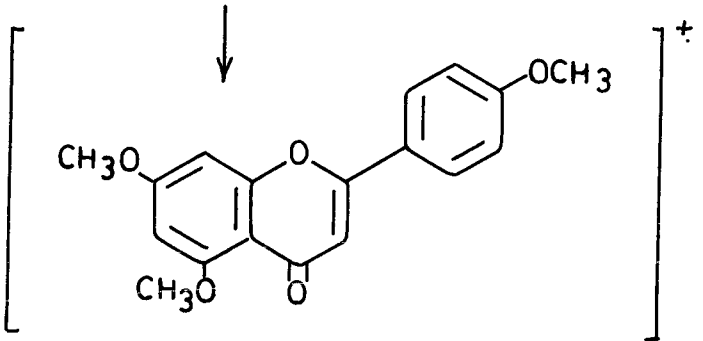

OCH3
B
CH2
m/e 121
CH3O
O
OCH3
XLI
M+, m/e 656
Electronbombardment and Thermal decomposition
m/e 164
m/e 154
RDA
ring
m/e 476
RDA at
C = O

SCHEME-III



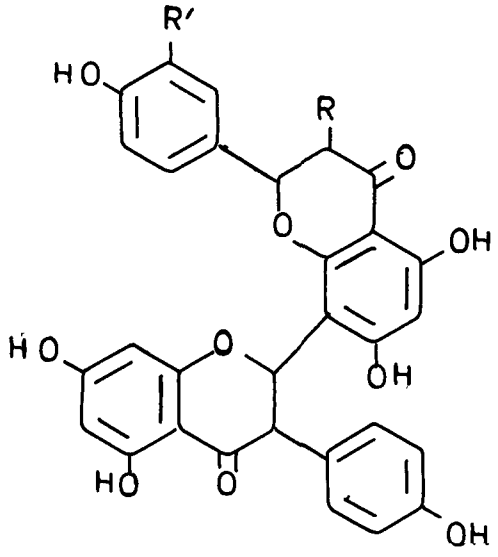
(XLI)

M^+, m/e 656



m/e 312

Pelter[8] further proposed another structure comprising of isoflavanone-flavanone units linked through 2-8" for biflavonoids of GB series (Va-d).



(V)

	R	R'
(a) GB-1	OH	H
(b) GB-1a	H	H
(c) GB-2	H	OH
(d) GB-2a	OH	OH

The chemical degradation would proceed equally well for compounds of the structures Va-d, this including the oxidation of GB-1 with iodine and sodium acetate to give a bisflavone type compound. Again NMR spectra would not be able to distinguish two type of compounds, in each case ring C being substituted at C-2 and C-3 by an aromatic ring. In the mass spectrum of GB-1a heptamethyl ether all the ions would be produced by the same process and would have the same structures with the exception of an ion at m/e 312 which would have an isoflavone type structure instead of a flavone type, these being indistinguishable in mass spectrometer. The only spectroscopic evidence that might have any bearing on the matter is the ion at m/e 121 in the mass spectrum of GB-1 methyl ether. If this is assumed to arise from C-2 of a flavanone, then its presence or absence in GB-2 heptamethyl ether might be indicative of the structure.[8]

The two stage breakdown fragmentation is fully substantiated by the presence of metastable peaks and fragmentation pattern below m/e 312 bears no resemblance to that found in apigenin measured under identical instrumental conditions[54]. The prominent ions at m/e 180 and 132 noted in the mass spectrum of apigenin trimethyl ether were entirely absent from the mass spectrum of GB-1 heptamethyl ether. This clearly indicates the unacceptability of Pelter's implications[54].

The formation of dimethyl phloroglucinol from the methyl ether derivative is probably of thermal origin. Infact, phloroglucinol is so readily lost from GB biflavanones that if the temperature of the ion chamber in the mass spectrometer much exceeds the minimum ($\sim 200^{o}$) for evaporation of the sample, there is difficulty in detecting the molecular ion. However, under all conditions the base peak was due to phloroglucinol[54].

Further the mass spectrum of GB-2 itself provided the answer to the objection of Pelter[8] as it clearly showed the presence of ions at m/e 107 and 123 consistent with the fragments obtained from aromatic rings B and E respectively[54].

Since it has been established that 4'-hydroxy flavanone loses a p-hydroxy benzyl fragment at C-2, it is perhaps significant that no peak consistent with a 2,4,6-trihydroxy benzyl moiety expected from the alternative structure suggested by Pelter[8] appears in any of the mass spectra of GB series[54].

On this basis, Jackson's structure i.e. flavanone-flavanone units linked together is preferable to the other structure i.e. isoflavanone-flavanone. Recent degradative studies on GB biflavonyls[55] further support the structure proposed by Jackson et al[7].

Thus the mass spectral studies of the biflavonyls

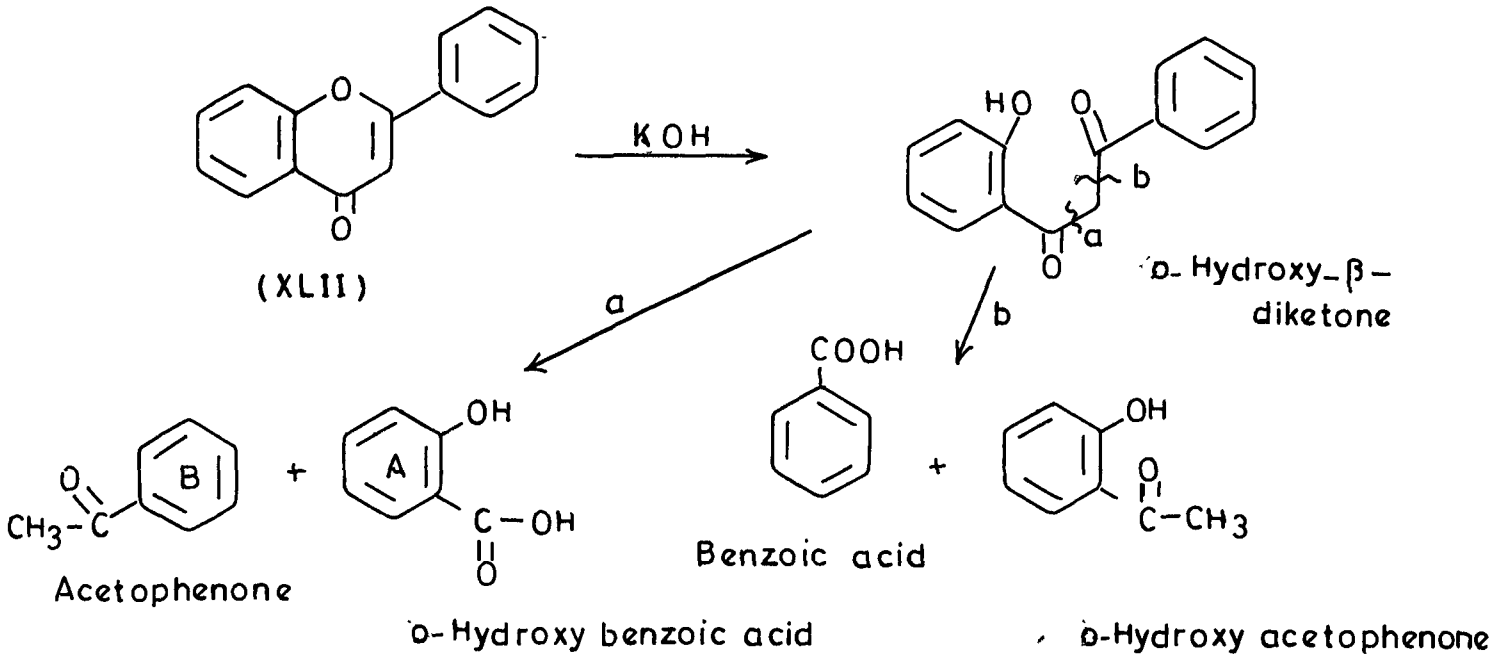
isolated from natural sources reveal that their fragmentation patterns depend not only on the constituent monomeric flavonoid units but also on the nature and the position of interflavonyl linkages. While the cracking patterns of simpler flavonoids are less complex, in application of these concepts to biflavonoids one has to take into consideration the influence of the additional structural and steric factors.

3. DEGRADATION

Degradation of biflavonyls can be brought about either by alkaline hydrolysis or oxidation with alkaline hydrogen peroxide.

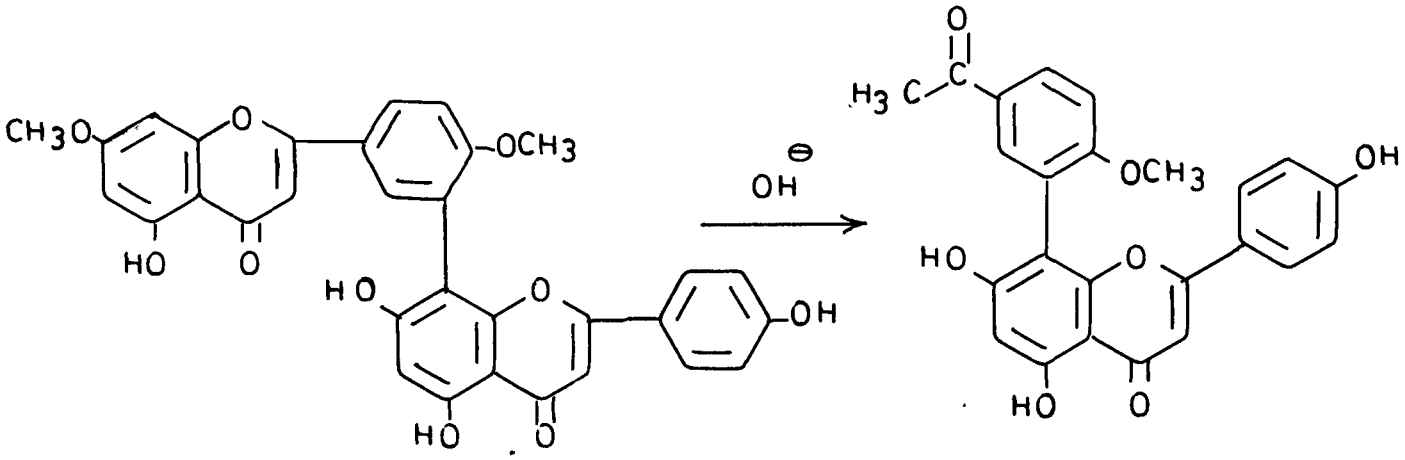
ALKALINE HYDROLYSIS

In general a flavone (XLII) gives four products which arise by opening of the pyrone ring followed by the fission of the intermediate o-hydroxy-β-diketone by two different paths (a) and (b). The intermediate can be isolated if



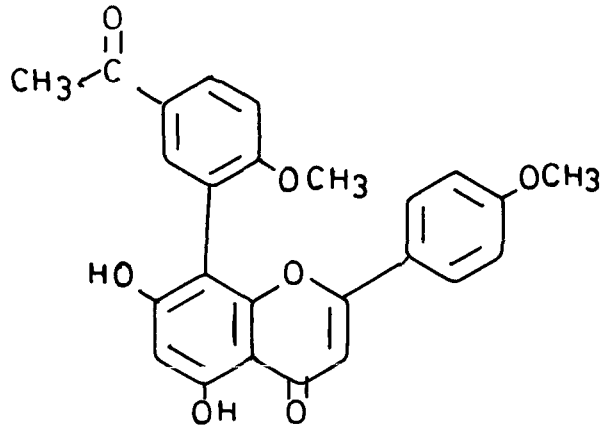
cold ethanolic solution of caustic soda is used.

In the case of biflavones, 'Ketoflavones' are characteristic degradation products of alkaline hydrolysis. Hydrolysis of ginkgetin (XLIII) by Kariyone and Kawano[56] gave a ketoflavone containing one methoxyl group, p-hydroxy acetophenone and 2,6-dihydroxy-4-methoxy acetophenone. IR spectrum of the ketoflavone showed two carbonyl frequencies (1658 and 1645 cm^{-1}) and hydroxyl absorption. As the location of the methoxyl group at C-4' in ginkgetin is already established, the ketoflavone according to spectral evidence must have the structure (XLIV).



(XLIII) (XLIV)

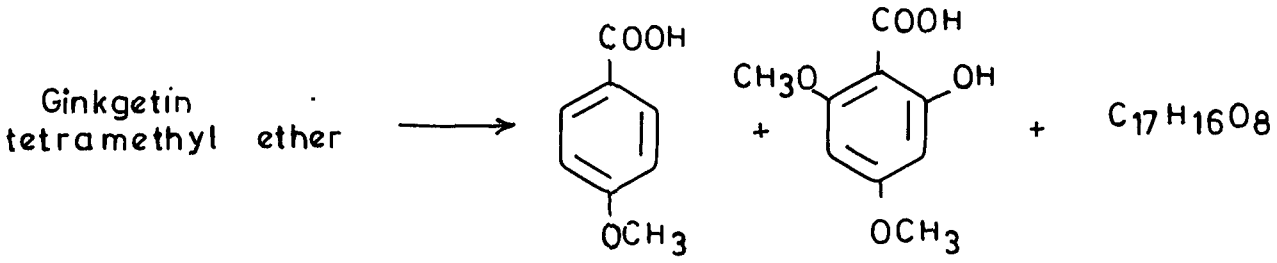
Alkaline hydrolysis of both isoginkgetin (Ig) and sciadopitysin (Ij) gave the same ketoflavone (XLV), thus supporting the structures proposed for these two biflavonyls[57].



(XLV)

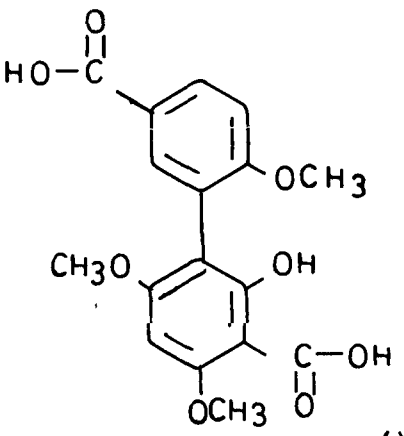
OXIDATION WITH ALKALINE HYDROGEN PEROXIDE

Alkaline hydrogen peroxide oxidation has been very helpful in the determination of the interflavonyl linkage. Ginkgetin tetramethyl ether on oxidation with alkaline hydrogen peroxide, gave anisic acid, 2-hydroxy-4,6-dimethoxy benzoic acid and a compound ($C_{17}H_{16}O_8$).

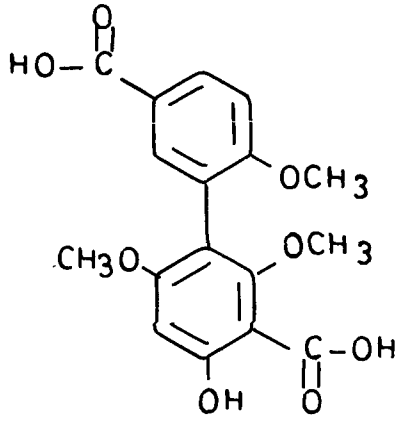


The compound ($C_{17}H_{16}O_8$) was shown to be a dicarboxylic acid, containing three methoxyl groups and one hydroxyl

group. To fit in all the spectral data, two structures (XLVI)and (XLVII) were postulated for the dicarboxylic acid.

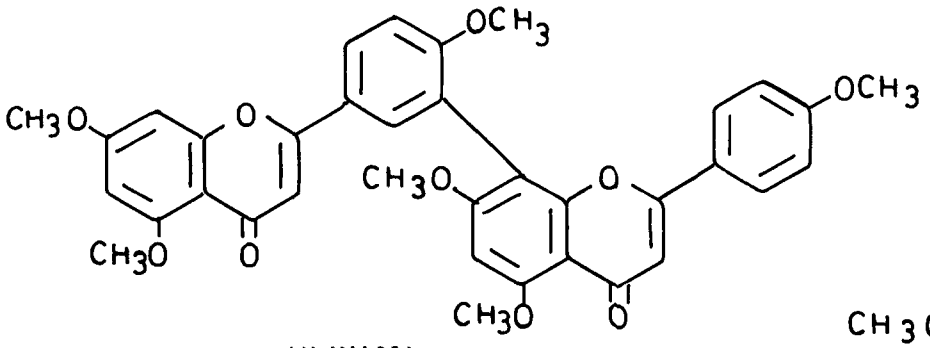


(XLVI)

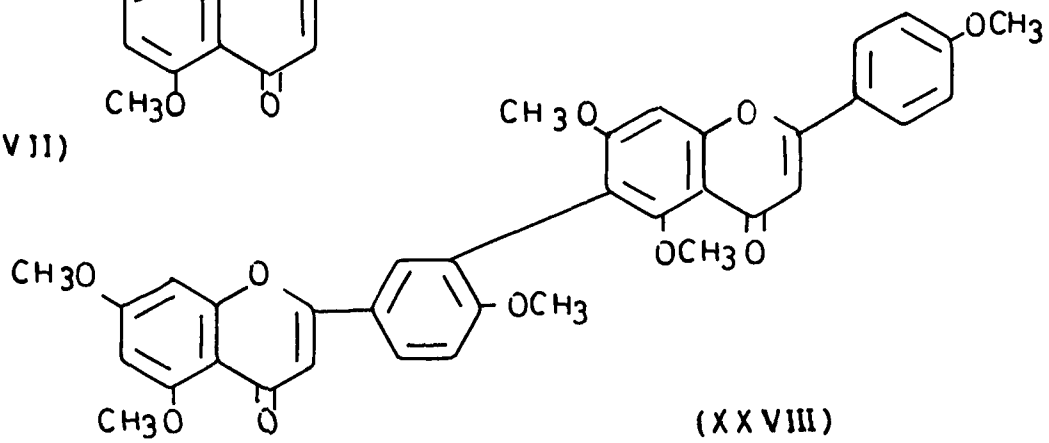


(XLVII)

These facts proved that a biphenyl residue must exist in ginkgetin molecule and that interflavonyl linkage must involve position 3' of one flavonoid residue and 6 or 8 of the other. The two structures (XXVII) and (XXVIII) for ginkgetin tetramethyl ether were,therefore,considered.



(XXVII)

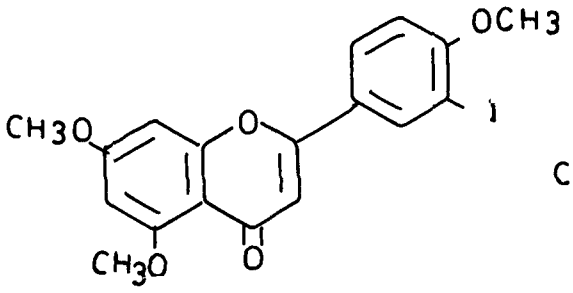


(XXVIII)

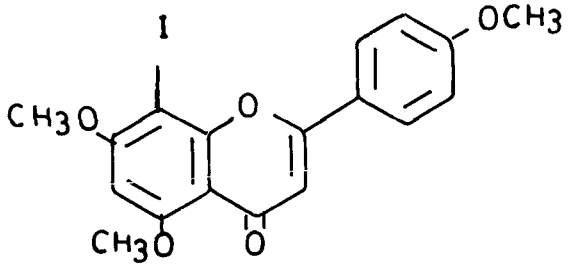
Of the two structures, one involving 3'-8" linkage was preferred. The other structure (3'-6") was considered unlikely since that 5"-OH in a compound with this structure would be severely hindered and there was no evidence that this hydroxyl group in ginkgetin was exceptionally difficult to methylate[39]. The structure with 3'-8" linkage (XXVII) was, therefore, proposed for ginkgetin tetramethyl ether and structure (XLVI) for the derived carboxylic acid.

4. SYNTHESIS

The first synthetic biflavonoid was reported by Mahesh and Seshadri[58], as a byproduct of the oxidation of acetophenone with Fenton's reagent in acid medium by dehydrogenative coupling in the 3-position. Afterwards a number of biflavonoids with different interflavonyl linkages have been synthesized by application of Ullmann reaction[59-61]. Nakazawa[62] accomplished the synthesis of amentoflavone hexamethyl ether by mixed Ullmann reaction between 3'-iodo-4',5,7-tri-O-methyl flavone (XLVIII) and 8-iodo-4',5,7-tri-O-methyl flavone (XLIX). Cupressuflavone hexamethyl ether was obtained as a byproduct and was found identical with the one obtained from natural sources. Later on Seshadri et al[5] have also synthesized cupressuflavone hexamethyl ether from 8-iodo-4',5,7-tri-O-methyl flavone (XLIX) under modified conditions of Ullmann condensation.

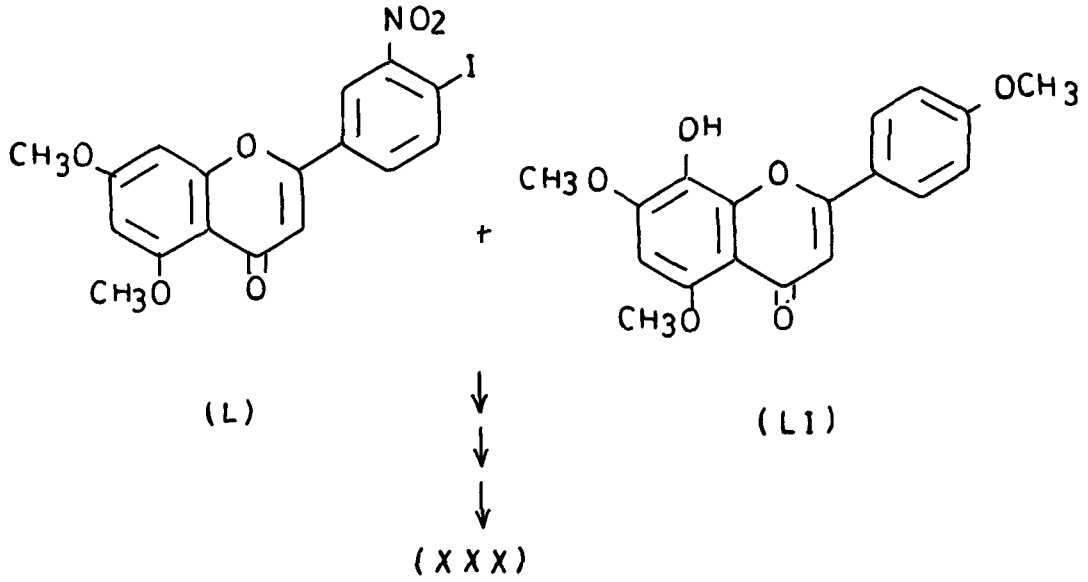


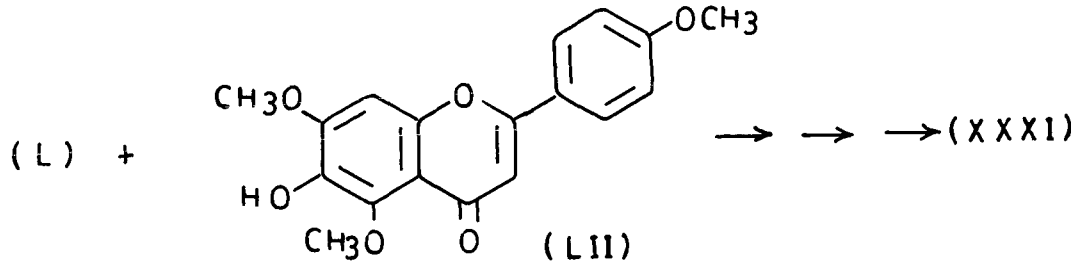
(XLVIII)



(XLIX)

However, in the key reaction demethylation occurred and a rearrangement to yield 6-6"/6-8" linked biflavones could not be ruled out. Recently 4'-O-8" and 4'-O-6" linked hinokiflavones have been synthesized by Nakazawa[42] in his elegant seventeen step synthesis. The permethylated 3'-nitrobiflavonyls, the key intermediates, were obtained by condensation of 3'-nitro-4'-iodo-5,7-di-O-methyl flavone (L) and 8- or 6-hydroxy-4',5,7-tri-O-methyl flavones (LI and LII) in DMSO in the presence of K_2CO_3. The nitro ethers were reduced by $Na_2S_2O_3$ in aqueous DMF, diazotized and decomposed with 50% H_3PO_2 to give pentamethyl ethers of hinokiflavones. (XXX and XXXI).





OXIDATIVE COUPLING OF PHENOLIC COMPOUNDS

It has long been recognised that a considerable diversity of structural types can be derived from the oxidation of phenols with such reagents as ferric chloride and potassium ferricyanide. In more recent years mechanistic organic chemistry has lent such vigour to the interpretation of biosynthetic processes that the oxidative utilization of phenolic substrates in biogenetic pathways, inferred earlier by inspection of the formulae of natural products[63], now seems to rest on a secure theoretical basis[64,65]. At the

same time, intensification of interest in molecular biochemistry has directed the organic chemists towards the synthesis of natural products by procedures which simulate certain steps of a proposed biosynthetic pathway. Recent progress in the application of phenol oxidation to synthetic chemistry has been due rather to this fresh analytical approach than to the discovery of new reagents and reactions.

It was the work of Pummerer and his collaborators[66] that first drew attention to the role of free radicals as intermediates in phenol oxidations. Experimental facts agreed with the view that the first step in the oxidation of monohydric phenol ArOH, by a one electron oxidant, is the generation of the phenoxy radical. The subsequent fate of the radical depends on the substitution pattern, but if oxidative dimerization of the molecule is assumed, three mechanisms[67] have to be considered viz. (i) homolytic coupling (ii) radical insertion and (iii) heterolytic coupling. These possibilities are illustrated for alkaline ferricyanide oxidation[67].

$$ArO^- + [Fe(CN)_6]^{3-} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ArO^{\cdot} + [Fe(CN)_6]^{4-}$$

(i) Homolytic coupling

$$2\ ArO^{\cdot} \longrightarrow (ArO)_2$$

(ii) Radical insertion

$$ArO^{\cdot} + ArO^{-} \xrightarrow{-e} (ArO)_2$$

(iii) Heterolytic coupling

$$ArO^{\cdot} \xrightarrow{-e} ArO^{+}$$

$$ArO^{-} + ArO^{+} \longrightarrow (ArO)_2$$

Although the second mechanism cannot be entirely disregarded, the intervention of radical insertion process in such phenol oxidations seems unlikely. Thus, it has been found[68] that one-electron oxidation of p-cresol in the presence of a large excess of veratrole affords no evidence of cross coupling.

Evidence for the intrusion of the cationic species ArO^{+} in these oxidations is also lacking. The inability of ArO^{+} to capture any nucleophile other than phenol anions as required by heterolytic mechanism offers some circumstantial evidence against this two-electron oxidation mechanism.

ESR studies of Waters and his coworkers[69] on the oxidation of catechol in alkaline solution has further supported the free-radical mechanism.

Thus at present, the best theory for phenol coupling mechanism is that the phenolate anion is oxidized by the reagent to a free radical. The free radicals are then coupled rapidly and irreversibly to dimeric and polymeric

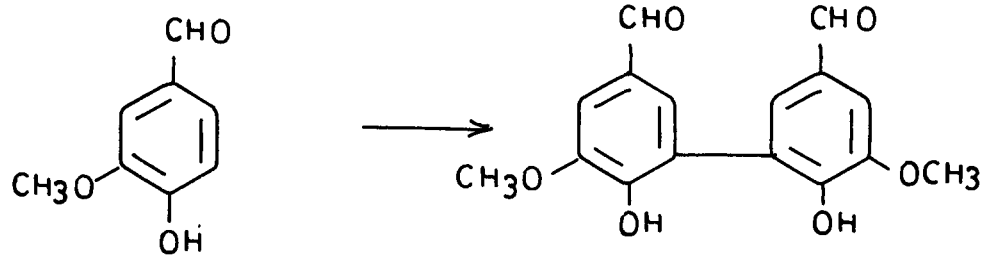
products under kinetic control. It is reasonable to assume that coupling occurs fastest at the positions of highest density of the free electron except where there is steric hinderance of approach.

The dimerization of phenols may involve carbon-carbon, carbon-oxygen or oxygen-oxygen bonding as illustrated in the following simple examples.

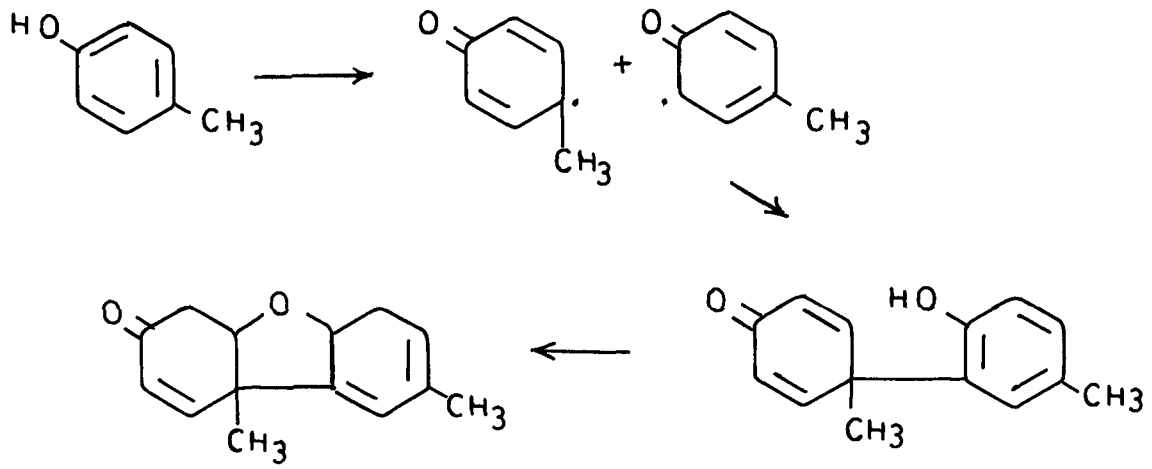
(a) <u>Carbon-Carbon coupling</u>

Para-Para

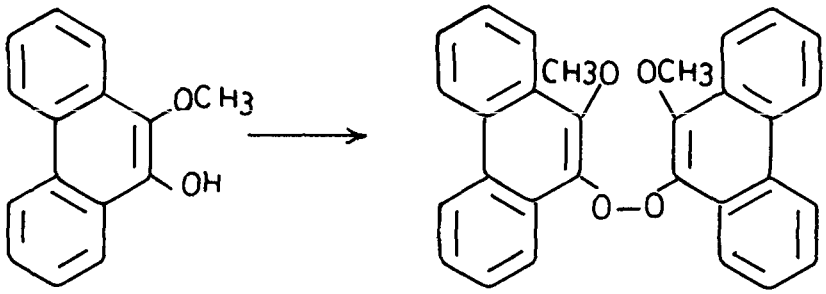
Ortho - Ortho



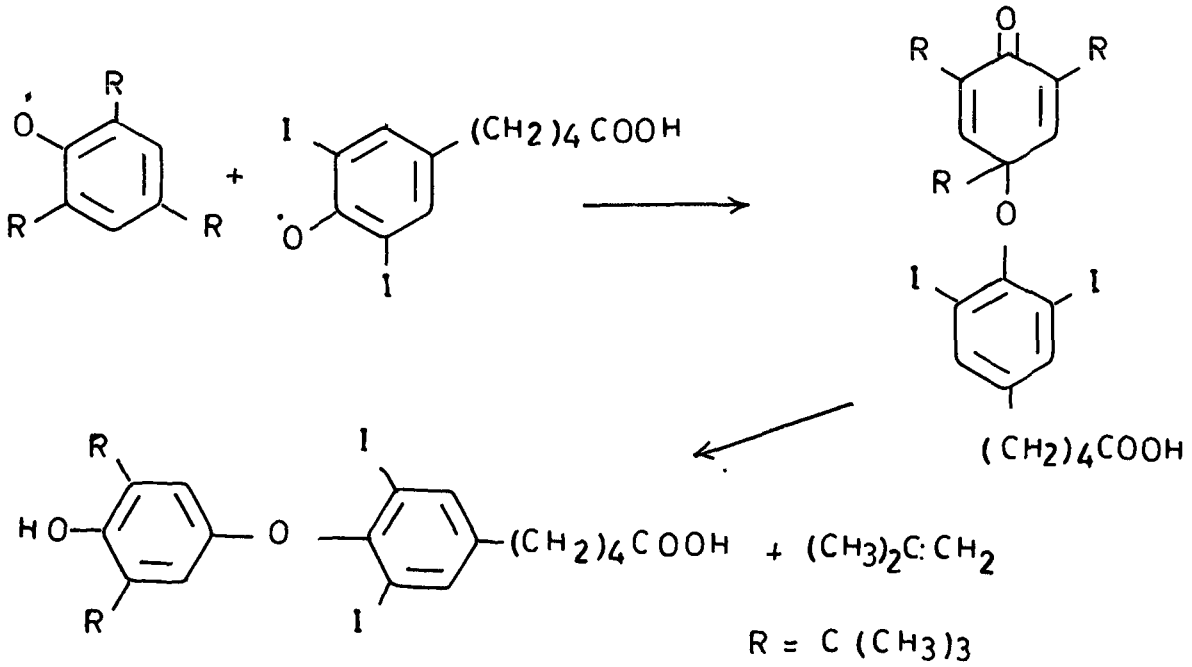
Ortho – Para



(b) Oxygen-oxygen coupling



(c) Carbon-oxygen coupling



Our knowledge of the structural features which influence the stability of free radicals is as yet insufficient to make it possible to anticipate in any but a few selected cases the actual course of intramolecular coupling when various alternatives are open.

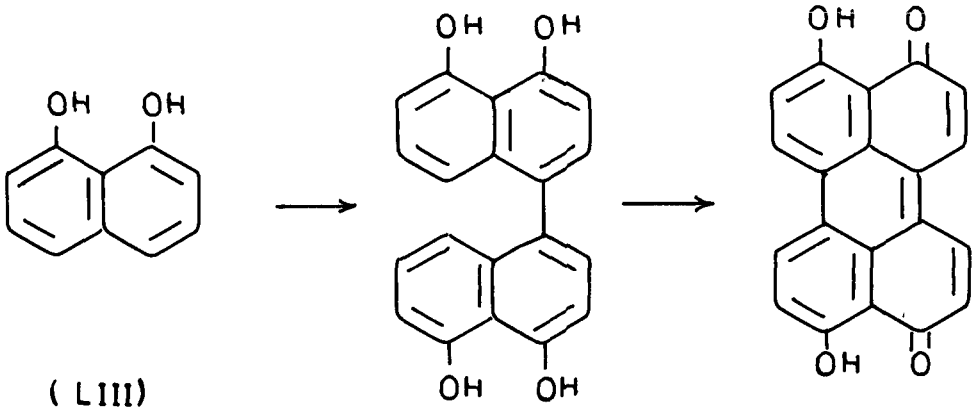
Notwithstanding recent intensification of efforts in this area, the selection of reagents and experimental conditions for a given substrate remains largely empirical. The most versatile reagents for oxidative coupling are alkaline ferricyanide and ferric chloride[64,65]. The former is to be preferred where metal-complex formation with either starting material or product becomes a serious consideration. On the other hand, ring cleavage of sensitive polyphenols e.g. pyrogallols, under alkaline conditions can preclude successful use of the former reagent. This undesired reaction can be eliminated by using selective protecting groups although radical activity may be limited by such expedients. The use of ferric chloride solution has proved successful in the catechol and pyrogallol series[70]. Oxidation of catechol, quinols and pyrogallols to the corresponding quinones is usually effected with silver oxide, ferricyanide, or tetrachloro-o-quinone[64,65,71]. Other oxidants which have been used in C-C, C-O, and C-N formation are mangnese[72] and lead dioxides[72], cerium (IV)[73] and vanadium (V)[73] salts, lead tetraacetate[64,65], and Fenton's reagent[64,65].

Several enzymes and cell-free extracts of higher plants have been found to catalyse the coupling of phenols. Most of these studies have been carried out with the system horse-radish peroxidase - hydrogen peroxide, usually in phosphate buffer[74]. Preliminary reports on the observation of free radicals in natural environment have also appeared[75].

Some examples of biogentic type synthesis involving C-C, C-O and O-O coupling are given below:

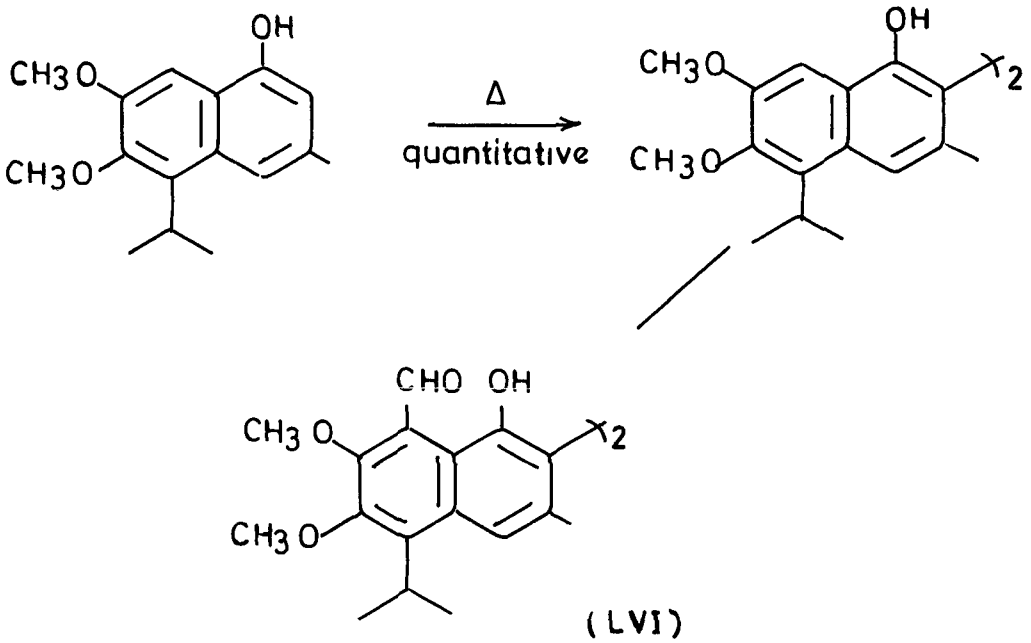
A. C-C Coupling:

(a) Bisnaphthols: The oxidation of 1,8-dihydroxy naphthalene has been studied in wild strains of Daldinia concentrica, where the metabolites (LIII) → (LV) are formed. Simulation of this process in the laboratory by ferricyanide oxidation of (LIII) gives first the bisnaphthol (LIV) and then the perylene-quinone (LV)[76].



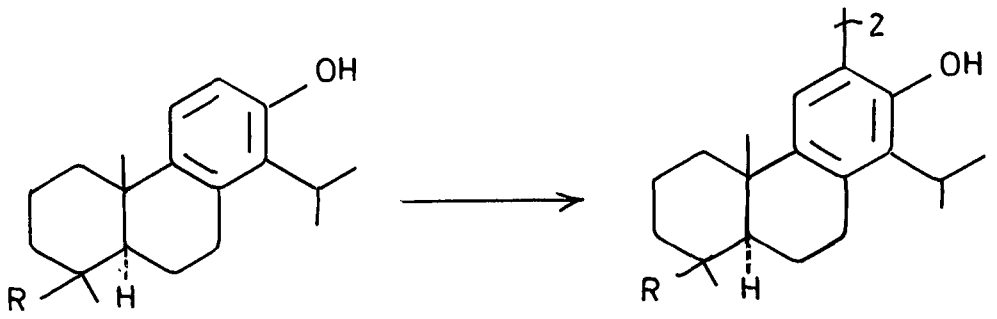
(LIII) (LIV) (LV)

(b) Bisterpenoids: An excellent example of symmetric C-C coupling of two naphthol units is evident in the structure of the bis-sesquiterpene gossypol (LVI)[77].



(LVI)

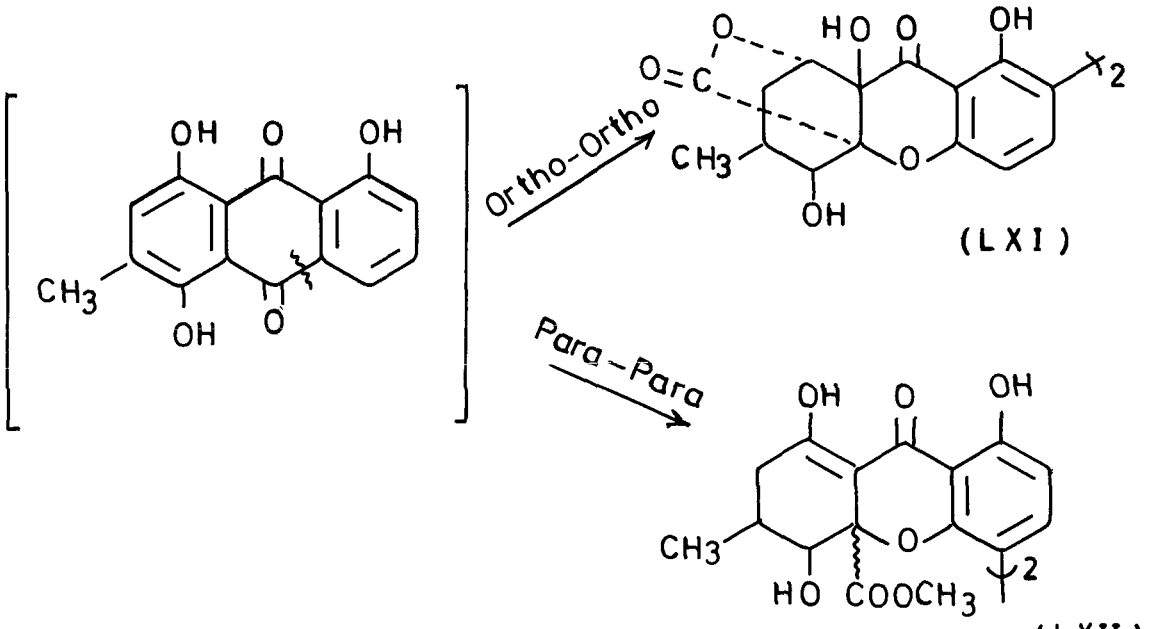
The preparation of some naturally occurring bisditerpenoids by phenol oxidative coupling has been achieved. Thus totarol (LVII) and the ester (LVIII) are coupled in presence of ferricyanide to podototarin (LIX) and macrophyllic acid (LX)[78,79] respectively.



(LVII) R = Me
(LVIII) R = COOEt

(LIX) R = Me
(LX) R = COOEt

Bisanthraquinones[79]: The operation of symmetric ortho and para coupling can be clearly discerned in the dimeric structures of ergot pigments ergoflavin (LXI) and secalonic acid (LXII), the monomeric units of which may be formed from anthraquinones as indicated[80].

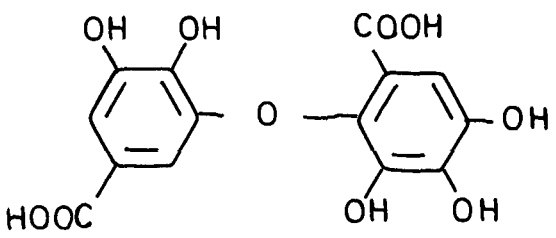


(LXI)

(LXII)

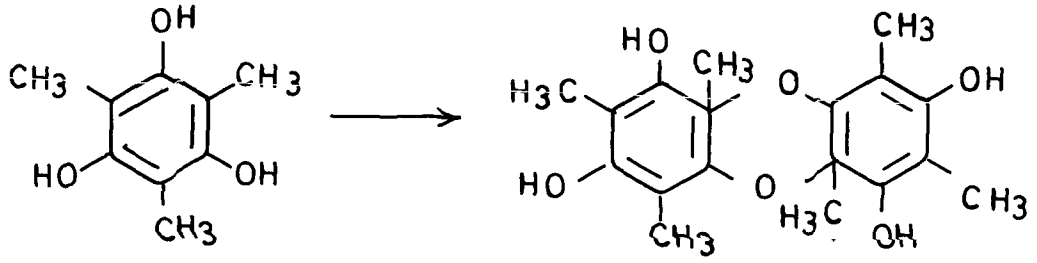
B. C-O Coupling

Dimerization through C-O bond formation becomes important only where steric hindrance precludes facile C-C bond formation. Several examples are known where C-O bond formation has occurred in vivo e.g. dehydrodigallic acid (LXIII).



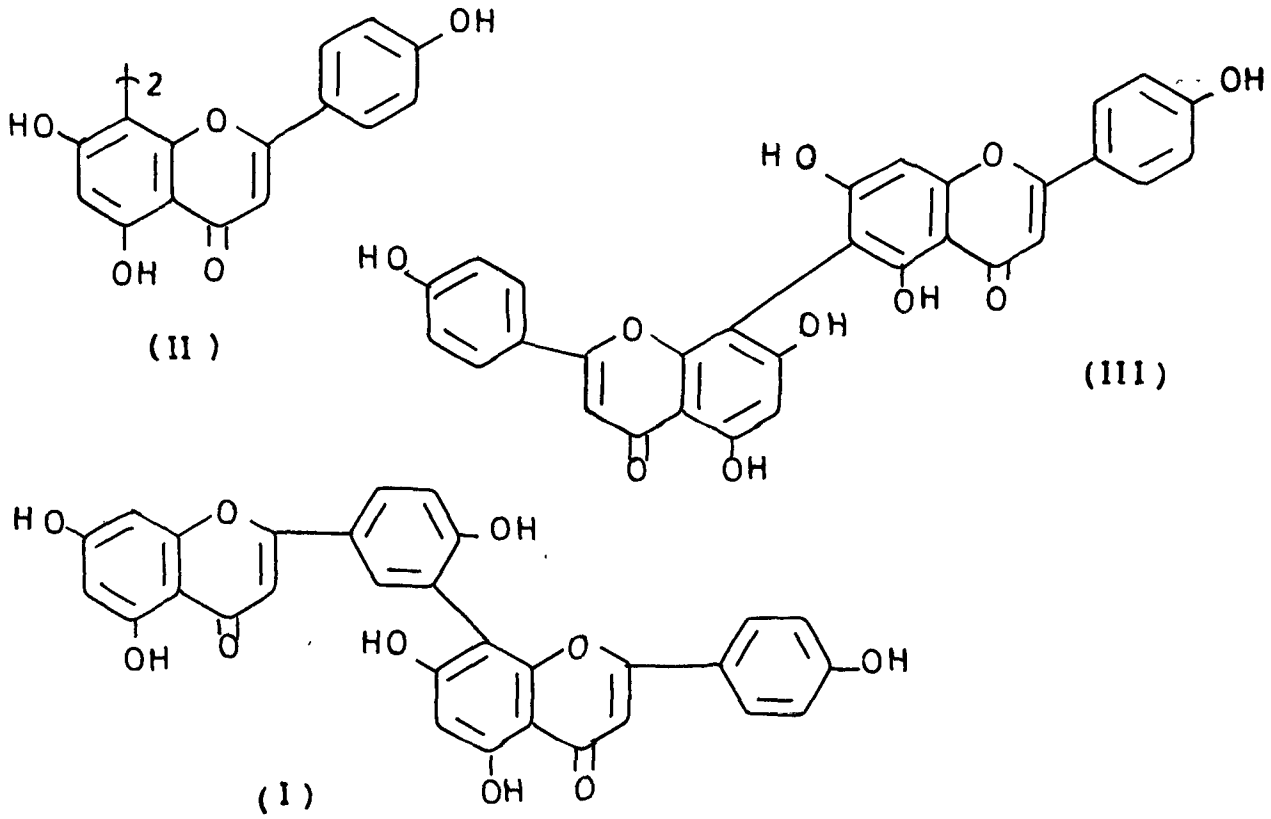
(LXIII)

Another interesting double oxidation can be inferred from the structure of cedrone (LXIV)[81].

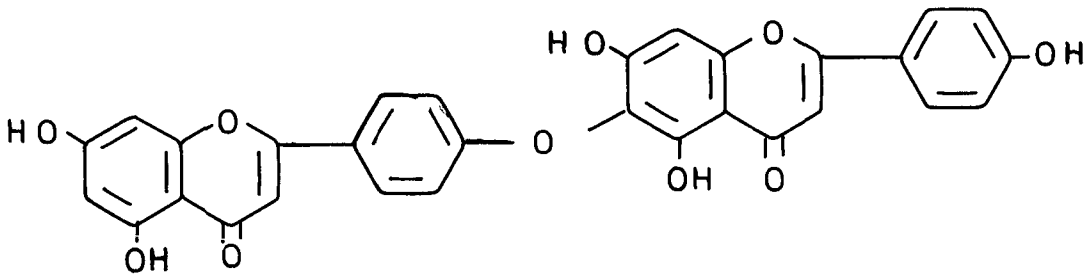


(LXIV)

Biflavonyls: The structures of biflavonyls (I, II, III, VII) strongly support the suggestion that their biosyntheses involve the oxidative coupling of free radicals (LXV) and (LXVI) derived either from apigenin (XXIII) or a closely related compound, in association with O-methylation[3]. This

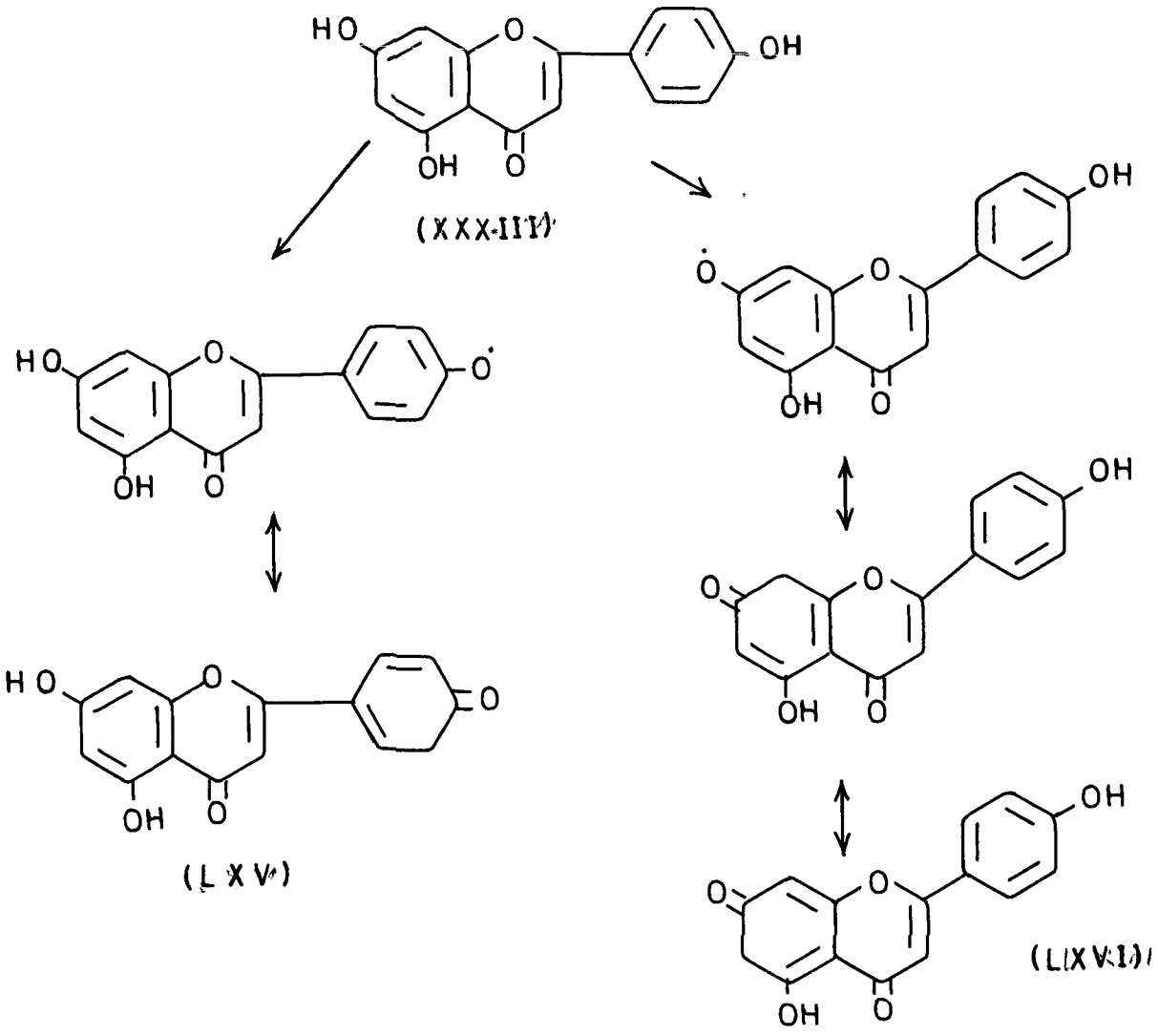


(II) (III) (I)



(VII)

restricts the interflavonyl linkages to positions 3', 6,8 giving six possible biflavonyl structures having 6-6, 6-8, 3'-6, 3'-8, 3'-3', and 8-8 carbon-carbon linkages. The



(XXXIII)

(LXV)

(LXVI)

isolation of biflavones, in which units of the same oxygenation pattern are 8-8", 3'-8", 6-8" and 4'-O-6" linked, from the members of Araucariaceae, further support the above postulate[6,82]. The unsymmetrical structure of amentoflavone does however suggest another possibility that it could arise as a result of an electrophilic attack of radical (LXV) upon apigenin or a derivative thereof at position 8. In this connection it may be noted that the radical (LXV) might be expected to be relatively more stable than (LXVI) and position 8 in apigenin is normally associated with electrophilic substitution. The formation of hinokiflavone(VII), a biphenyl ether type biflavonyl probably involves a related oxidative coupling leading to C-O-C linkage.

D I S C U S S I O N

BIFLAVONYLS FROM GUTTIFEREAE

The biflavonyls are restricted mainly to the leaves of Gymnosperms. The occurrence of these pigments in Angiosperms is exception[39] rather than a rule. Out of the three families (Angiosperms) examined so far[7,9,10,55,83,84], Guttifereae has relatively been more extensively investigated[7,9,10,55,83]. The striking features of this family are the occurrence of these pigments in heartwood and bark and that they represent biflavonyls comprising of reduced heterocyclic systems. The present discussion deals with our investigations on the phenolic extractives of leaves, heartwood and bark of Garcinia livingstonii. The plant material was procured from Horticulture Research Center, Saharanpur (U.P.).

Biflavonyls from leaves of Garcinia livingstonii:

The fact that no mention seems to have been made regarding the occurrence of biflavonyls in the leaf extracts of plants belonging to Garcinia, attracted our attention and prompted us to undertake the present investigation. The phenolic extractives of defatted leaves by solvent fractionation and column chromatography followed by preparative thin-layer chromatography (silica gel) yielded two components. After establishing their homogeneity (TLC) they were obtained in fine crystalline forms. The usual colour reactions, ultra violet spectra in ethanol and with various added diagnostic

reagents indicated both of them to be flavonoids. The components were labelled as WG-1 and WG-2. Both the components on methylation gave the same methyl ether (WG-3). The structures of these pigments have been fully elucidated by UV, IR, NMR and mass spectral studies. These components constitute the first example of the presence of flavone-flavone type biflavonyls in Garcinia species.

4',4"',5,5",7,7"-Hexahydroxy-3',8"-biflavone (WG-1):

WG-1 parent	m.p. 225-56°	R_F = 0.17	M.Wt. 538
WG-1 acetate	m.p. 235°	,, ,,	790
WG-3(methyl ether)	m.p. 225°	R_F = 0.404 ,,	622

The mass spectrum of WG-3 showed a base peak at m/e 622 (M^+). There were various losses of 15, 28 etc. but the significant peak was at m/e 311, indicating that each flavonoid unit had three methoxy groups. Using double irradiation technique it was possible to assign τ value to each proton with the exception of H-3 and H-3" which could not be distinguished. The results of NMR studies of WG-3 are shown in fig. III and table IV.

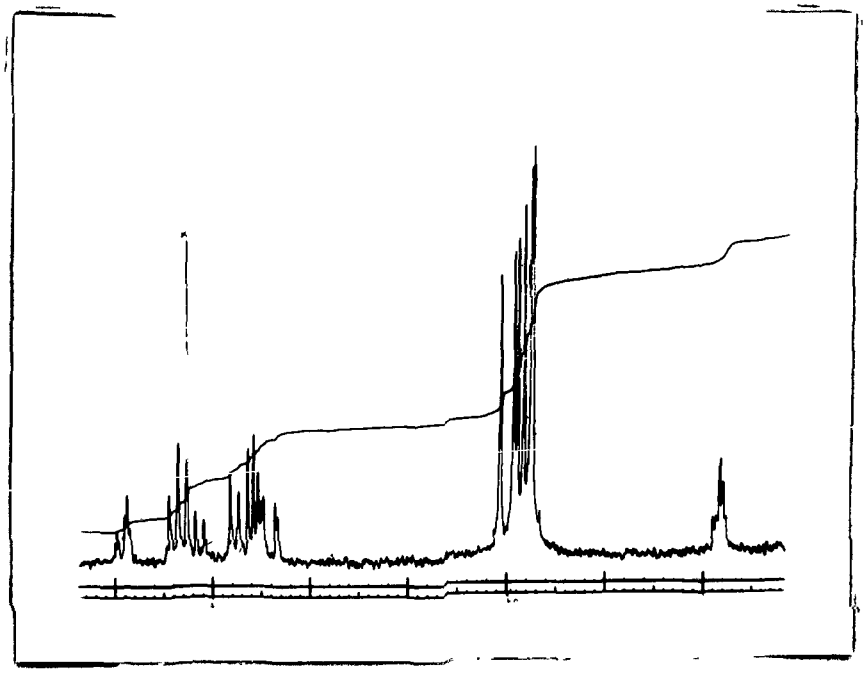


Fig. III

T A B L E - IV

Chemical shifts of protons of WG-3*

Signal	No. of protons	J c/s	Assignment
3.52 d	1	3	H - 8
3.66 d	1	3	H - 6
3.38 s	1	-	H - 6"
2.10 q	1	J_1 = 8 J_2 = 3	H - 6'
2.88 d	1	8	H - 5'
2.16 d	1	3	H - 2'
2.62 d	2	9	H-2"', 6"'
3.24 d	2	9	H-3"', 5"'
3.48 s 3.42 s	2	-	H-3, 3"
5.94, 6.12	6	-	OMe-5,5"
6.08, 6.18	6	-	OMe-4"', 7"
6.25, 6.27	6	-	OMe-4', 7

s = singlet, d = doublet, q = quartet

*spectrum run in $CDCl_3$ at 100 M c/s, TMS as internal standard = τ 10.00.

Associated with rings B and E, there were evidenced ABX and A_2 B_2 systems. Thus rings B and D of the biflavone seemed to be involved in interflavonyl linkage. In particular, the values showed that C-3' was linked to C-6" or C-8". The observation that in flavones, having an aromatic substituent at C-8, the 5-methoxy group generally appears below τ 6.00 (Table V) led us to believe that substituent (flavone unit) in WG-3 was located at C-8" and not at C-6".

T A B L E - V

Biflavonyl	5-OMe	5"-OMe
Cupressuflavone hexamethyl ether	τ5.88	τ5.88
Amentoflavone hexamethyl ether	τ6.13	τ5.94
Agathisflavone hexamethyl ether	τ6.41	τ5.95
Hinokiflavone (4'-O-8") pentamethyl ether	τ6.00	τ5.92
WG-3	τ6.12	τ5.94

Further all methoxy groups on change of solvent from deuterochloroform to benzene moved upfield showing that every methoxy group had at least one ortho proton and therefore a C-8" rather than C-6" linkage was confirmed.

An authentic sample of ($\pm$) amentoflavone hexamethyl ether[2] was shown to give an NMR identical with that of WG-3 in all respects including solvent dependent methoxy shifts.

The IR, UV and mass spectra were also identical. Thus both the compounds (WG-1 and WG-2) belong to the amentoflavone series.

The results of NMR studies of WG-1 hexaacetate are shown in table VI.

T A B L E - VI

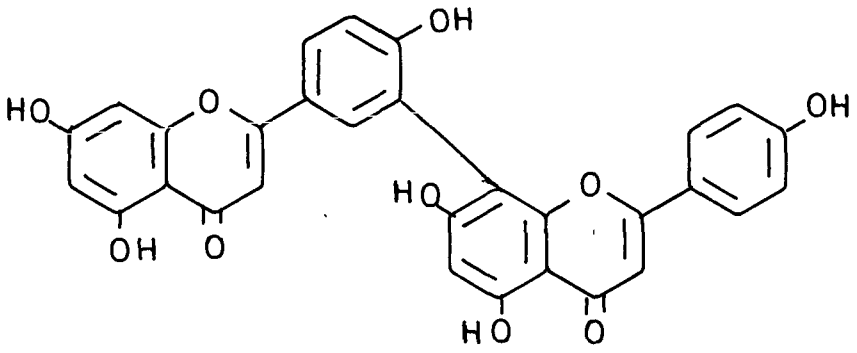
Chemical shifts of protons of WG-1 hexaacetate*

Signal	No. of proton	J c/s	Assignment
2.73 d	1	3	H - 8
3.31 d	1	3	H - 6
2.97 s	1	-	H - 6"
1.99 q	1	$J_1 = 8$ $J_2 = 3$	
2.48 d	1	9	H - 5'
1.94 d	1	3	H - 2'
2.92 d	2	9	H - 3", 5"'
2.50 d	2	9	H - 2"', 6"'
3.30 s 3.32 s	2	-	H - 3, 3"
7.50, 7.54	6	-	OAc - 5, 5"
7.89, 7.93	6	-	OAc - 4',4"'
7.67, 7.72	6	-	OAc - 7,7"

s = singlet, d = doublet, q= quartet

*spectrum run in $CDCl_3$ on 100 M c/s, TMS as internal standard = τ 10.00.

Thus the component WG-1 was assigned the structure of 4',4"',5,5",7,7"-hexahydroxy-3',8"-biflavone(amentoflavone, Ia).



(Ia)

<u>4"'-O-Methyl-4',5,5",7,7"-pentahydroxy-3',8"-biflavone (WG-2)</u>:

WG-2	parent	m.p. 266-68°	R_F 0.37	M.Wt. 552
WG-2	acetate	m.p. 253-54°	-	M.Wt. 762.
WG-3	(methyl ether)	m.p. 225°	R_F 0.404	M.Wt. 622

Mass spectrum of WG-2 acetate (M^+, m/e 762; base peak at m/e 552) showed it to be a monomethoxy pentaacetate of amentoflavone.

The possibility of methoxy group being at 5 or 5" position was ruled out as in the parent compound itself there were two hydrogen bonded hydroxy groups at τ-2.56 and τ-3.07, expected for these positions. The absorption maxima appeared at 275 nm (Band-I)and 335 nm (Band-II).

Addition of N/50 NaOEt caused a bathochromic shift of band-I with an increase in intensity and of band-II with a moderate decrease in intensity thus indicating that no methoxy group was present at C-7 or C-7". The only position left for methoxy group might be 4' or 4"'. As B_2 protons of A_2 B_2 pattern of ring E were found almost invariant in both WG-2 acetate and WG-3 (Table VII) the methoxy group *was assigned to C-4"'*.

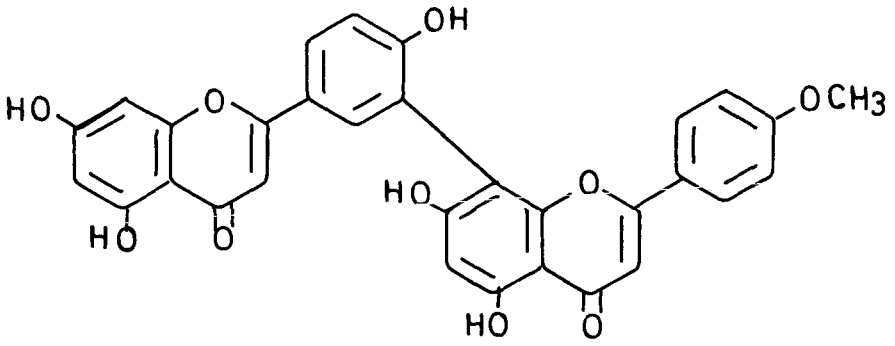
T A B L E - VII

Proton	Signal in(τscale)	
	WG-2 acetate	WG-3
H - 2'	1.95 d	2.16 d
H - 5'	2.49 d	2.88 d
H - 6'	2.00 q	2.10 q
H-2"', 6"'	2.58 d	2.60
H-3"', 5"'	3.21 d	3.24

The appearance of ions at m/e 132 and 135 consistent with $[C_6H_4(OMe).C{\equiv}CH]^+$ and $[C_6H_4(OMe)CO]^+$ respectively in the mass spectrum of WG-2 further supported the above assignment.

WG-2 was, therefore, assigned the structure of 4"'-O-methyl-4',5,5",7,7"-pentahydroxy-3',8"-biflavone

(podocarpusflavone A, Ie).



(Ie)

Further confirmation to the above structure was provided by the IR spectrum of WG-2, which was found superimposable with that of an authentic sample[2].

The results of NMR studies of WG-2 acetate are given in table VIII.

T A B L E - VIII

Chemical shifts of protons of WG-2 pentaacetate*

Signal	No. of protons	J c/s	Assignment
2.75 d	1	3	H - 8
3.15 d	1	3	H - 6
3.01 s	1	-	H - 6"
1.95 d	1	3	H - 2'
2.49 d	1	9	H - 5'
2.00 q	1	J_1 = 9 c/s J_2 = 3 c/s	H - 6'
2.58 d	2	9	H - 2"', 6"'
3.21 d	2	9	H - 3"', 5"'

T A B L E - VIII (Contd.)

Signal	No. of protons	J c/s	Assignment
3.40 s 3.42 s	2	-	H - 3, 3"
6.25	3	-	OMe - 4"'
7.68	3	-	OAc - 4'
6.51, 7.56	6	-	OAc - 5, 5"
7.90, 7.94	6	-	OAc - 7, 7"

s = singlet, d = doublet, q = quartet
*spectrum run in $CDCl_3$ as 100 M c/s, TMS as internal standard = τ 10.00.

The differences in m.ps. of the parent compounds and those reported in literature[2] (Table IX) appear to be due to the racemic nature of the earlier samples and the optical activity of the materials now isolated.

T A B L E - IX

Biflavonyl	melting points		$[\alpha]_D^{25}$
	optically active	racemic	
Amentoflavone	255-56°	> 300°	+ 9
Podocarpus-flavone A	266-68°	320-23°	- 6

Biflavonyls from heartwood and bark of Garcinia livingstonii

The acetone extracts of the deffated heartwood on solvent fractionation and repeated preparative thin-layer chromatography (silica gel; benzene: pyridine: formic acid, 36:9:5, as developing system) yielded two closely spaced homogeneous components labelled as BGH-II and BGH-III (R_F values 0.023 and 0.211 respectively). The present discussion deals with the spectral and chemical evidences in support of the structures assigned to these components.

3"',4',4"',5,5",7,7"-Heptahydroxy-3(8"-) flavonyl flavanone (BGH-II):

BGH-II (parent)	m.p.	300°	R_F 0.023
BGH-II (acetate)	m.p.	212-15°	-
BGH-II (methyl ether)	m.p.	212-13°	R_F 0.395

The pigment gave a red colour in the Shinoda test[12]. IR spectrum showed broad hydroxyl bands at 3250 cm^{-1} and chelated carbonyl at 1645 cm^{-1}. The latter resolved into two bands at 1670 and 1645 cm^{-1} in the spectrum of BGH-II methyl ether. The appearance of a carbonyl band at higher frequency and the other remaining unchanged in methyl ether indicated the presence of 5-hydroxy flavanone and 5-hydroxy flavone structures in BGH-II. UV spectrum showed maxima at 255, 273, 290 and 345 nm. The molecular formula determined by mass

spectrometry, and analytical methods corresponded to $C_{30}H_{20}O_{11}$ and was in agreement with the above suggestions.

BGH-II on methylation with methyl iodide and potassium carbonate yielded a methyl ether (M^+, 654; $C_{37}H_{34}O_{11}$). The mass spectrum supported the presence of phloroglucinol nucleus derived from 5,7-dihydroxy flavanone system with ions at m/e 154 and 181 consistent with fragments $\lceil C_6H_3(OMe)_2.OH \rfloor^{\cdot+}$ and $\lceil C_6H_2(OMe)_2.OH.CO \rfloor^{\cdot+}$ respectively. In addition, the presence of another free aromatic ring in flavanone unit was suggested by ions at m/e 121 and 108 consistent with fragments $\lceil C_6H_4(OMe).CH_2 \rfloor^{\cdot+}$ and $\lceil C_6H_5.OMe \rfloor^{\cdot+}$ respectively. The existence of an ion at m/e 162 $\lceil C_6H_3(OMe)_2.C{\equiv}CH \rfloor^{\cdot+}$, indicated the non-implication of ring B of flavone unit in the inter-flavonyl linkage.

NMR spectrum of the methyl ether showed methoxy signals between τ 6.08 to τ 6.36 which integrated for seven methoxyls. The doublets at τ 4.16 and 5.08 (J_{trans} = 12 c/s) were shown to be coupled by double resonance. These were assigned to H-2 and H-3 trans protons of ring C of the flavanone unit. Aromatic protons showed signals between τ 2.6 and τ 3.9. Protons of ring E gave rise to double doublets (or quartet) at τ 2.6 (J_{ortho} = 9 c/s, J_{meta} = 3 c/s), a doublet (J_{meta} = 3 c/s) at τ 2.85 and another (J_{ortho} = 9 c/s) at τ 3.2, these signals together forming an ABC pattern. The H-2',6' and H-3',5' protons of ring B gave rise to two doublets (J_{ortho} = 9 c/s) at τ 2.91 and τ 3.40 respectively. Two meta coupled

doublets (J = 3 c/s) at τ 3.88 and 3.81 were assigned to H-8 and H-6 protons of ring A. Of the two singlets at τ 3.74 and 3.54, the former was assigned to H-6" and the latter to H-3" (olefinic proton) by an analogy with 5,7-dimethoxy flavone[25a] where H-8, H-6 and H-3 showed τ values 3.47, 3.64 and 3.38 respectively. Results of NMR studies of BGH-II methyl ether are given in table X.

T A B L E - X

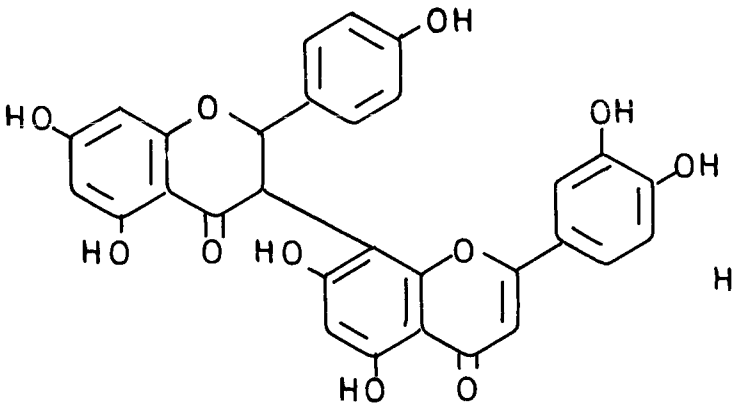
Chemical shifts of protons of BGH-II methyl ether*

Signals	No. of protons	J c/s	Assignment
2.91 d	2	9	H - 2', 6'
3.40 d	2	9	H - 3', 5'
2.6 q	1	J_1=9 J_2=3	H - 6"'
2.85 d	1	3	H - 2"'
3.20 d	1	9	H - 5"'
4.16 d	1	12	H - 2
5.08 d	1	12	H - 3
3.81 d	1	3	H - 8
3.88 d	1	3	H - 6
3.74 s	1	-	H - 6"
3.54 s	1	-	H - 3"
6.08 - 6.36	21	-	7 OMe

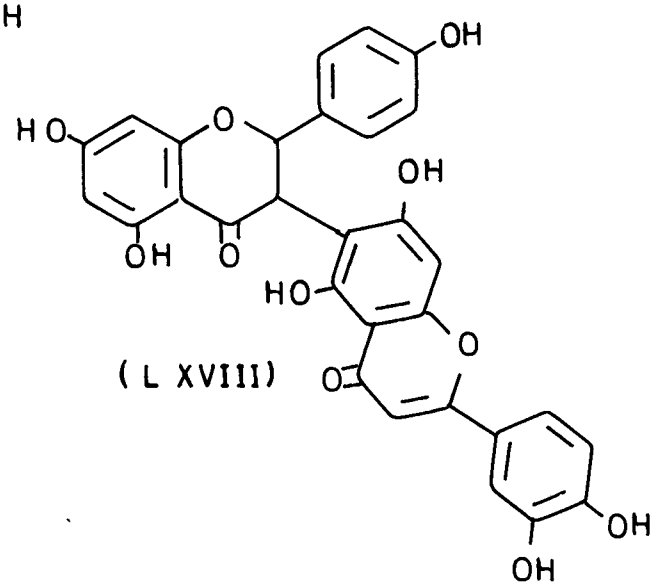
s = singlet, d = doublet, q= quartet.

*spectrum run in $CDCl_3$ at 100M c/s, TMS as an internal standard = τ 10.00.

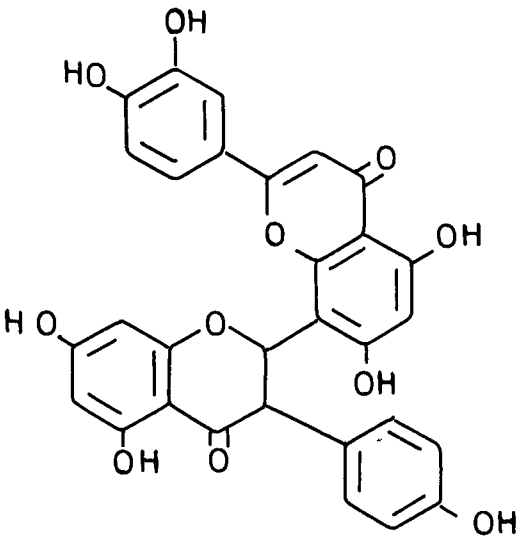
The data was well consistent with the structures (LXVII, and LXVIII) in which C-3 of flavanone unit is linked with the C-8 or C-6 of the flavone unit. The possibility of an isoflavanone-flavone structure with 2-8"/2-6" linkage (LXIX and LXX) could not be ruled out at this stage as in the NMR spectrum of methyl ether the aliphatic protons at C-2 and C-3 both having an aromatic substituent were expected to show the same chemical shifts as in flavanone-flavone structures (LXVII and LXVIII). The mass spectrum supported



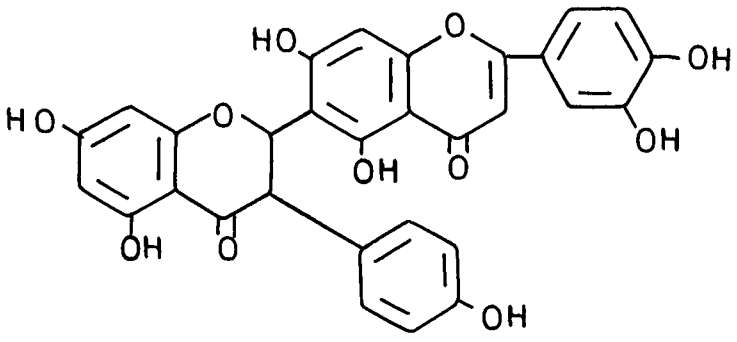
(LXVII)



(LXVIII)



(LXIX)



(LXX)

the 3-8"/3-6" type of linkage (structures LXVII and LXVIII) since the fragmentation pattern of the molecule ion at m/e 654 could be rationalised by RDA of flavanone at ring C to give a fragment ion at m/e 474 followed by loss of 28 units (CO) from pyrone ring F (flavone unit) to give ion at m/e 446. These results could only be accommodated by a linkage from the heterocyclic ring C (flavanone unit) to the phloroglucinol ring D (flavone unit).

The problem of implication of C-8 or C-6 of the flavone unit in the interflavonyl linkage was solved by studying the solvent induced shifts of methoxy resonances[6,27,40,41]. On change of solvent from $CDCl_3$ to C_6D_6 all the methoxy resonances (τ 6.08 - 6.36) moved upfield (τ 6.5 - 6.8) (Fig. IV) showing that each methoxy group had at least one ortho proton. This clearly fixed the flavanone substituent at C-8 (LXVII) rather than at C-6 (LXVIII) in the flavone unit.

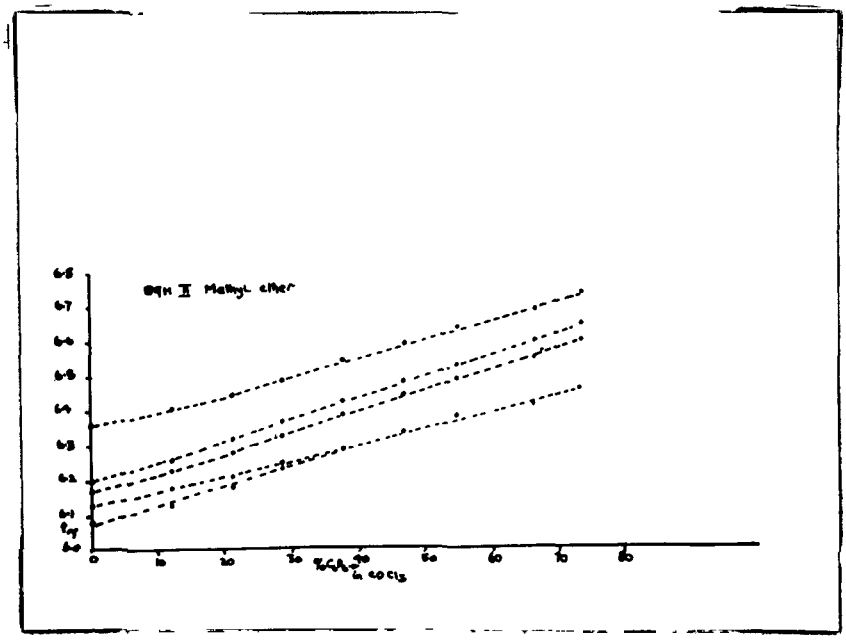


Fig. IV

On acetylation with pyridine and acetic anhydride, BGH-II gave an acetate, m.p. 212-15°. The most interesting observation was the complete lack of correlation between the NMR spectra of BGH-II acetate and its methyl ether. The doublets due to H-2 and H-3 protons of ring C had disappeared and instead a singlet appeared at τ 3.92. Further the signals due to acetoxy groups integrated for 24 protons instead of expected 21 protons. It seemed as if during the acetylation procedure, opening of the pyrone ring C had occurred transforming flavanone to the corresponding chalcone. Although such cases of isomerization of flavanone to chalcone during acetylation with acetic anhydride in the presence of sodium acetate in monomeric flavanoids are already reported[85,86] the flavanone-chalcone transformation using pyridine-acetic anhydride for acetylation in the present case appeared unusual, more so as only the latter conditions are claimed to be most satisfactory for the preparation of flavanone acetate[87]. Another striking feature of the compound was its non-isomerization during methylation using dimethyl sulphate/ methyl iodide and potassium carbonate in acetone. It is worthy of mention that methylation under aforesaid conditions is known to induce chalcone formation[87].

The signal at τ 3.92 was assigned to H-β of the chalcone unit. The results of NMR studies of BGH-II acetate are given in table XI.

T A B L E - XI

Chemical shifts of protons in BGH-II acetate*

Signal	No. of protons	J c/s	Assignment
2.47 d	2	9	H - 2, 6
3.01 d	2	9	H - 3, 5
2.15 q	1	J_1 = 9 J_2 = 3	H - 6"'
2.68 d	1	9	H - 5"'
2.15 d	1	3	H - 2"'
3.40 d	1	3	H - 3'
3.52 d	1	3	H - 5'
3.21 s	1	-	H - 6"
3.92 s	1	-	H- β
3.38 s	1	-	H - 3"
7.26, 7.67, 7.72(6H), 7.78(6H), 7.80, 8.08.	24	-	8 OAc

s = singlet, d = doublet, q = quartet.

*spectrum run in $CDCl_3$ at 100 Mc/s, TMS as an internal standard = τ 10.00.

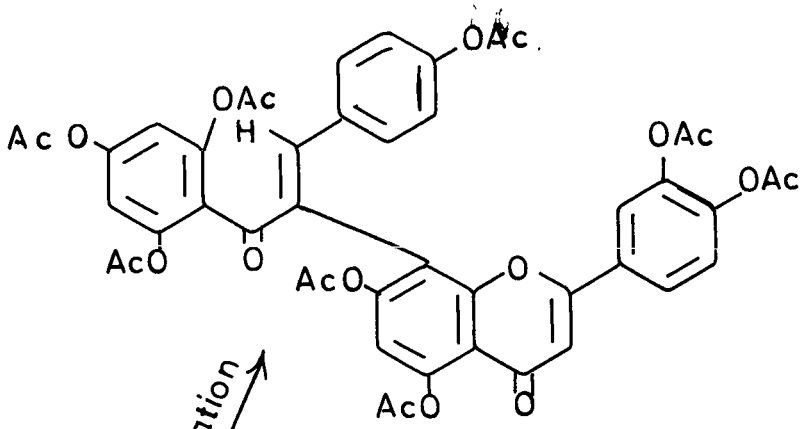
Protons of an acetoxy group appeared at a considerably higher field, τ 8.08 than the other acetoxyl protons. It may be attributed to the phenomenon of interatomic diamagnetic shielding. This shielded acetoxy group may

be either at C-7 of ring D which is close to the unsaturated system of chalcone or at C-4 of ring B which may be under the influence of the ring current of ring E in flavone half of the molecule.

In order to confirm that the interflavonyl linkage is at C-3 (flavanone-flavone structure) and not at C-2 (isoflavanone-flavone structure) the BGH-II acetate was subjected to ozonolysis. The formation of p-acetoxy benzaldehyde (detected by TLC) clearly indicated the 3-8" linkage and ruled out the alternative possibility.

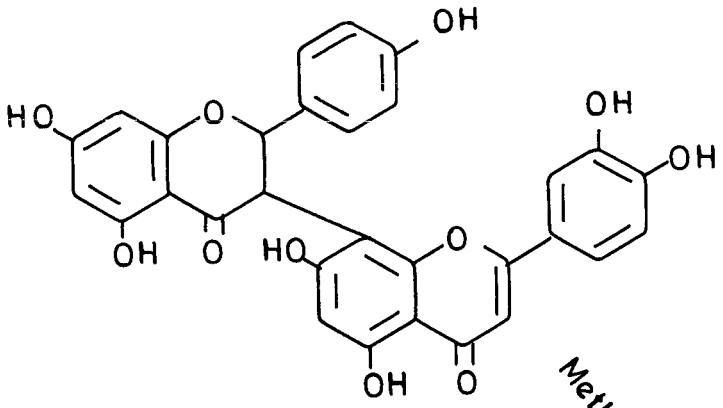
Mass spectral studies of BGH-II methyl ether (LXXII) (Scheme IV) provided additional support to 3(8"-) flavonyl flavanone structure. The fragmentation pattern was in full agreement with the previous observations on the mass spectra of flavanoids[48-54].

Considering all the above facts BGH-II and its acetate were assigned the structures 3"', 4', 4"', 5, 5", 7, 7"-heptahydroxy-3(8"-) flavonyl flavanone (LXVII)[9] and 2', 3"', 4, 4', 4"', 5", 6', 7"-octaacetyl-α(8"-) flavonyl chalcone (LXXI).



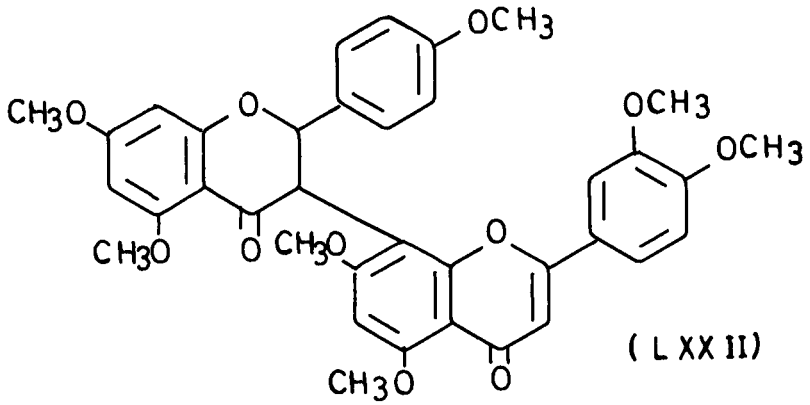
(LXXI)

Acetylation



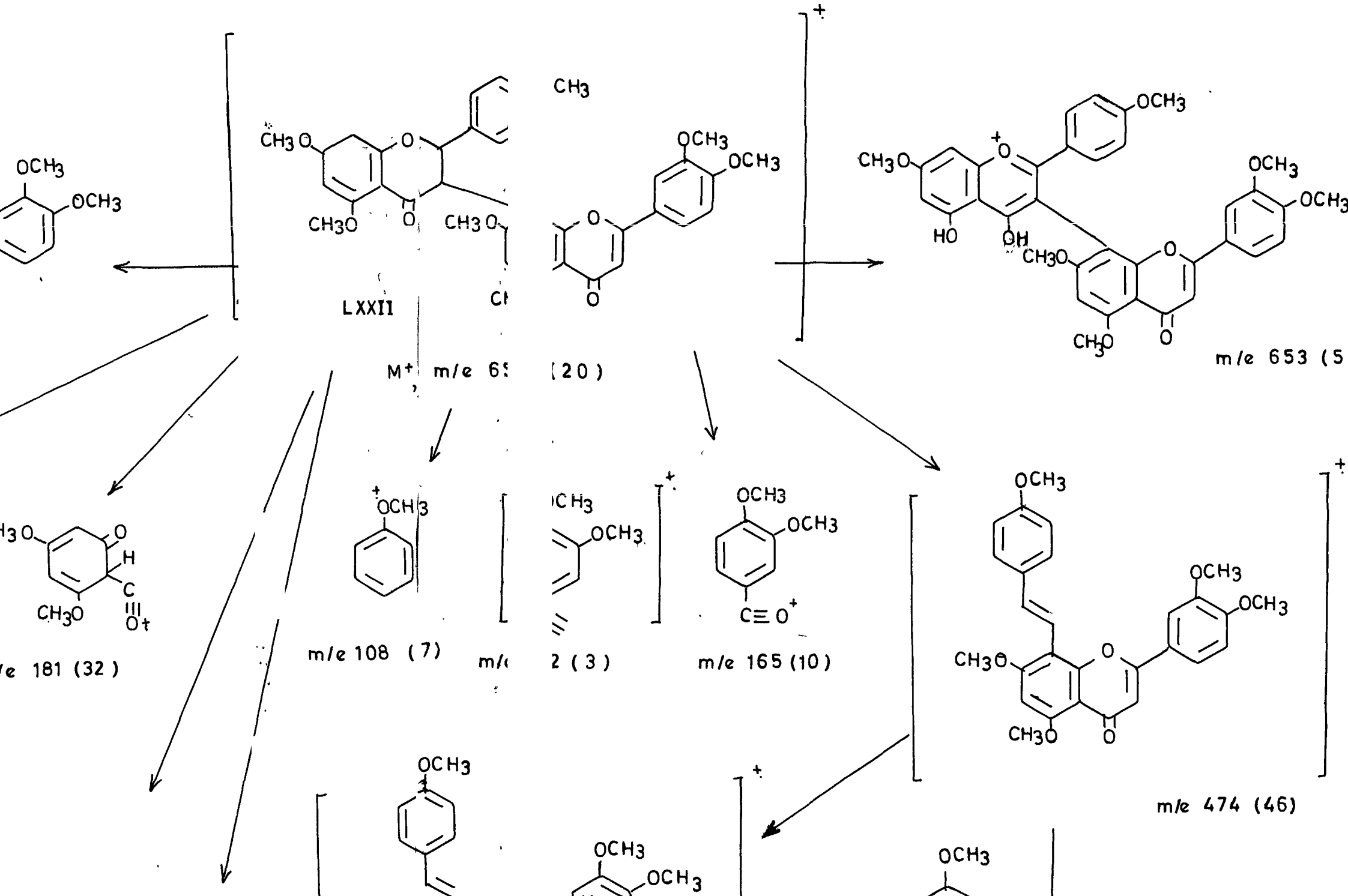
(LXVII)

Methylation



(LXXII)

SCHE IV



4',4"',5,5",7,7"-Hexahydroxy-3(8"-) flavonyl flavanone (BGH-III):

BGH-III	(parent)	m.p. 267-68°	R_F 0.211
BGH-III	(acetate)	m.p. 202-05°	-
BGH-III	(methyl ether)	m.p. 254-55°	R_F 0.420

The pigment gave a strong red colour in Shinoda test[12]. IR spectrum showed broad hydroxyl band at 3250 cm^{-1} and chelated carbonyl at 1645 cm^{-1}. In methyl ether the latter was resolved into two peaks at 1670 and 1645 cm^{-1} thereby indicating the presence of both 5-hydroxy flavanone and flavone units in the molecule. The molecular formula determined by mass spectrometry and analytical methods was found to be $C_{30}H_{20}O_{10}$ and was in agreement with the above suggestions.

BGH-III on methylation yielded a methyl ether (M^+, 624; $C_{36}H_{32}O_{10}$) m.p. 254-56°. The mass spectrum supported the presence of phloroglucinol nucleus derived from 5,7-dihydroxyflavanone system with ions at m/e 154 and 181 consistent with fragments $[C_6H_3(OMe)_2OH]^+$ and $[C_6H_2(OMe)_2OH.CO]^+$ respectively. In addition the presence of another free aromatic ring in flavanone unit was suggested by ions at m/e 121 and 108 consistent with $[C_6H_4(OMe).CH_2]^+$ and $[C_6H_5OMe]^+$ respectively. The existence of an ion at m/e 132 $[C_6H_4(OMe)\,C{\equiv}CH]^+$

indicated the non-implication of ring B of flavone unit in the interflavonyl linkage.

NMR spectrum of the methyl ether showed a multiplet at τ 6.08 - 6.36. The multiplet integrated for 18 protons (six methoxyls). Double resonance experiments showed that doublet at τ 4.28 (J=12 c/s) was coupled to the doublet at τ 5.18. These signals were attributed to the H-2 and H-3 trans protons of ring C of the flavanone unit. The aromatic protons were assigned (double resonance) as shown in table XII.

T A B L E - XII

Chemical shifts of protons of BGH-III methyl ether*

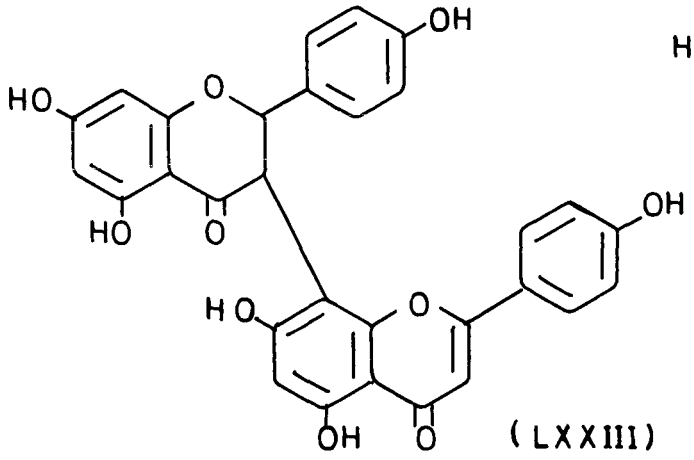
Signal	No. of protons	J c/s	Assignment
2.95 d	2	9	H - 2', 6'
3.44 d	2	9	H - 3', 5'
2.4 d	2	8	H - 2"', 6"'
3.2 d	2	8	H - 3"', 5"'
3.7 d	1	2	H - 8
3.9 d	1	2	H - 6
3.82 s	1	-	H - 6"
3.59 s	1	-	H - 3"
4.28 d	1	12	H - 2
5.18 d	1	12	H - 3
6.08, 6.13, 6.17(6H), 6.20, 6.36.	18	-	6 OMe

s = singlet, d = doublet.

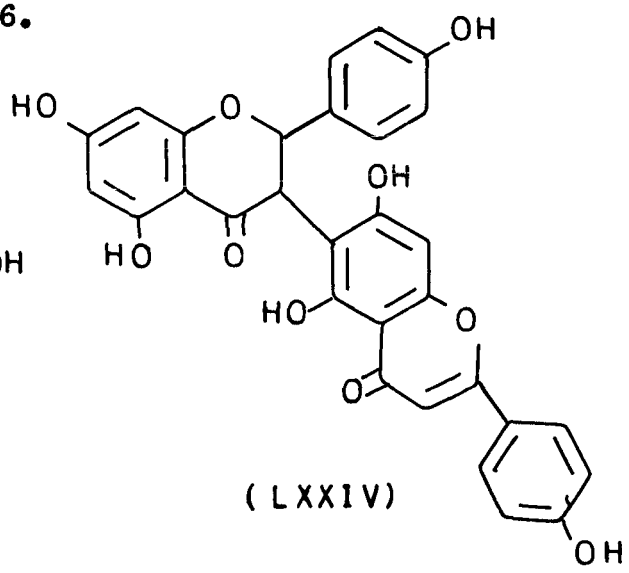
*spectrum run in $CDCl_3$ at 100 M c/s, TMS as an internal standard = τ 10.00.

The two sets of aromatic protons of A_2 B_2 pattern were assigned to rings B and E. The two meta coupled aromatic signals at τ 3.7 and 3.9 were attributed to protons of phloroglucinol ring A. Of the two singlets at τ 3.82 and 3.59, the former was assigned to H-6" (ring D) and the latter to H-3" (olefinic proton in ring F).

The data was in full agreement with the structures (LXXIII and LXXIV) in which C-3 of a flavanone unit is linked either to C-8 or C-6.



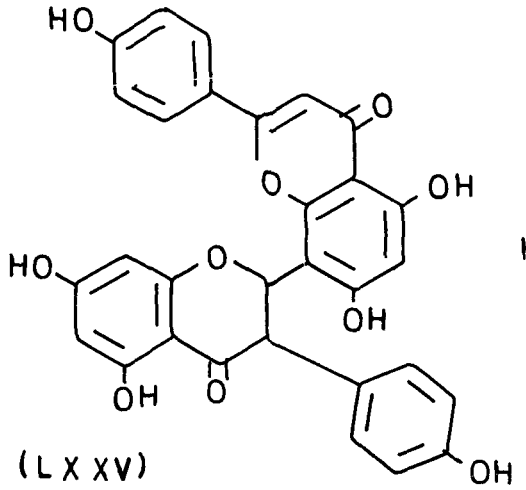
(LXXIII)



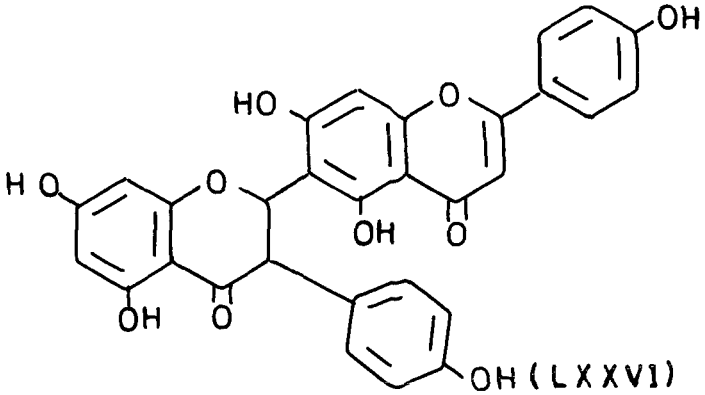
(LXXIV)

The mass spectrum supported the nature of linkage since the fragmentation of the molecule ion at m/e 624 could be rationalized by RDA of the flavanone at ring C to give a fragment ion at m/e 444 followed by loss of 28 units (CO) from pyrone ring F of flavone unit to give ion at m/e 416. These results could only be accommodated by a linkage from the heterocyclic ring C (flavanone unit) to the phloroglucinol ring D (flavone unit). The possibility of .. isoflavanone-

flavone structures (LXXV and LXXVI) with 2-8"/2-6" linkage was ruled out by ozonolysis of its acetate as described in the case of BGH-II.



(LXXV)



(LXXVI)

The problem of linkage with C-6 or C-8 of ring D was solved by solvent induced shift studies of methoxyl resonances[6,27,40,41]. On change of solvent from $CDCl_3$ to C_6D_6 all the methoxy resonances (τ 6.08-6.36) moved upfield (τ 6.5 - 6.8) (Fig. V) showing that each methoxy group had

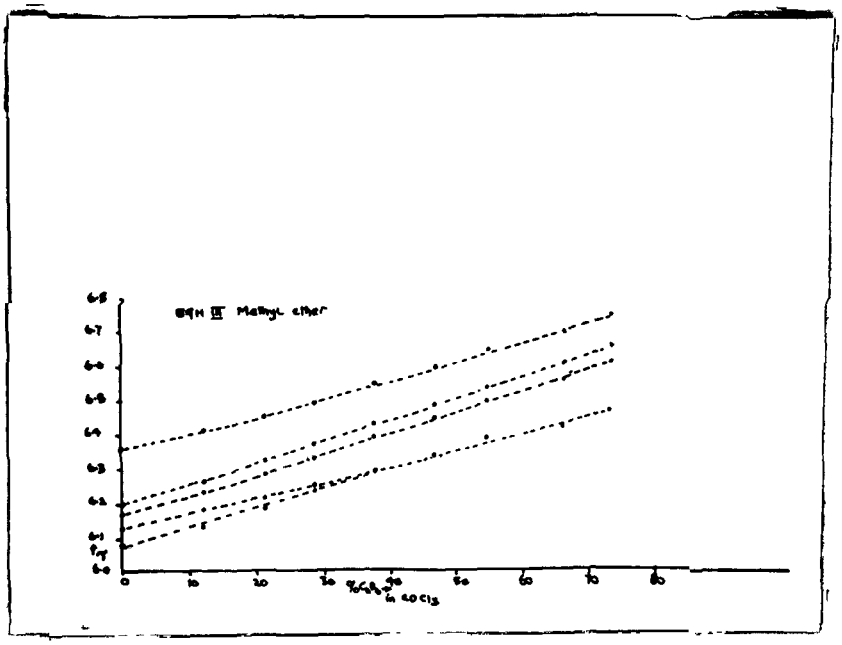


Fig.V

one ortho proton. This clearly established the 3-8" rather than 3-6" linkage.

Acetylation of BGH-III with pyridine-acetic anhydride gave an acetate, m.p. 202-05°. Here again there seemed to be a complete lack of correlation between NMR spectra of its methyl ether and acetate. The doublets at τ 4.28 and 5.18 due to H-2 and H-3 protons had disappeared and instead a down field singlet appeared at τ 3.92. Further the signals due to acetoxyl protons integrated for 21 protons instead of expected 18 protons. This observation based on flavanone-chalcone isomerization during acetylation has been fully explained in the case of BGH-II. The singlet at τ 3.92 was assigned to H-β of the chalcone unit. The results of NMR studies of BGH-III acetate are shown in table XIII.

T A B L E - XIII

Chemical shifts of protons of BGH-III acetate*

Signal	No. of protons	J c/s	Assignment
2.45 d	2	9	H - 2, 6
3.00 d	2	9	H - 3, 5
1.98 d	2	8	H - 2"', 6"'
2.75 d	2	8	H - 3"', 5"'
3.38 d	1	2	H - 3'
3.49 d	1	2	H - 5'
3.22 s	1		H - 6"

TABLE - XIII (Contd.)

Signal	No. of protons	J c/s	Assignment
3.36 s	1	-	H - 3"
3.92 s	1	-	H - β
7.62, 7.69, 7.70, 7.72, 7.78(6H), 8.08	21	-	7 OAc

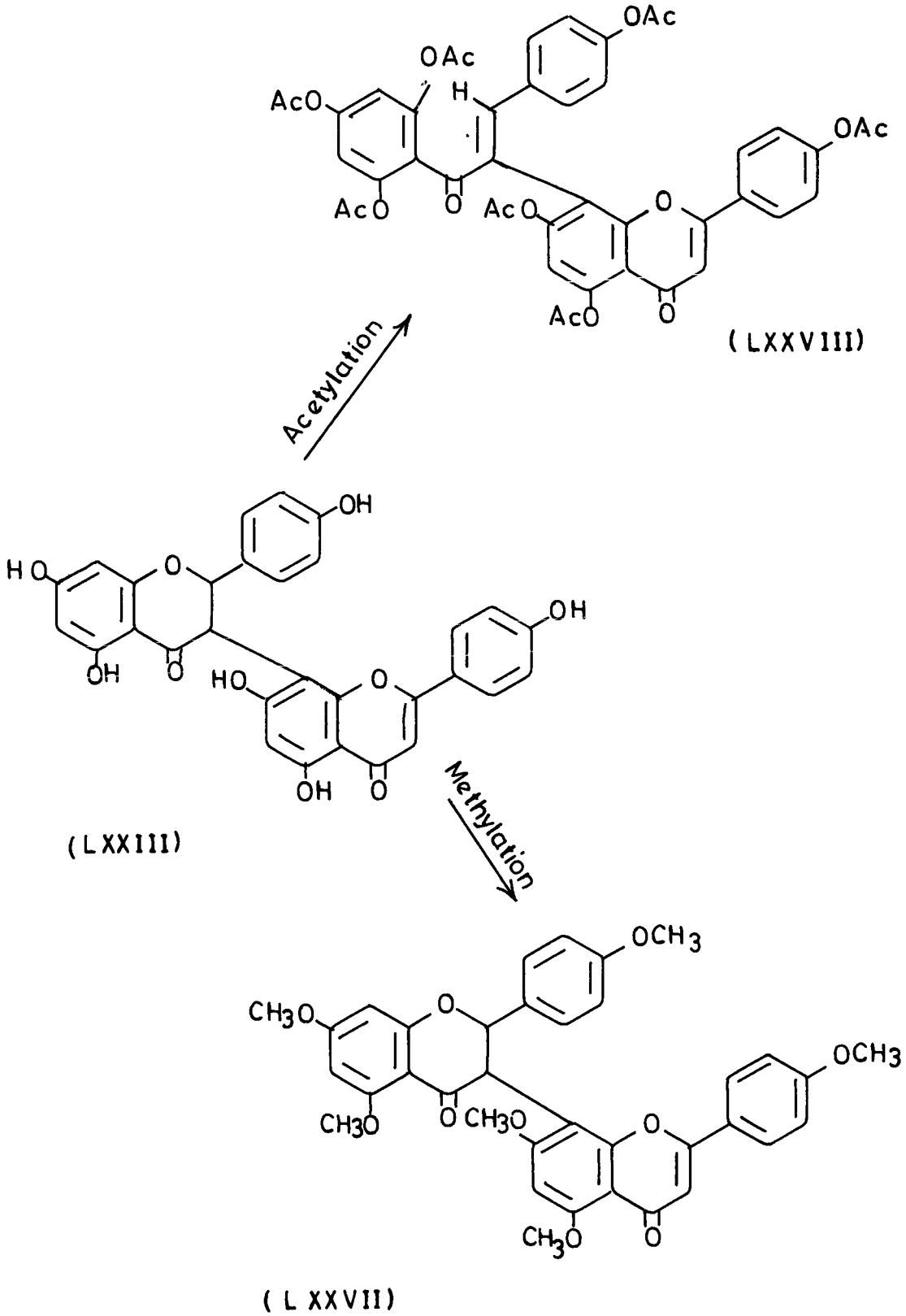
s = singlet, d = doublet.

*spectrum run in $CDCl_3$ at 100 M c/s, TMS as an internal standard = τ 10.00.

Protons of an acetoxy group appeared at a considerably higher field than the other acetoxyl protons. It seemed to be a phenomenon of interatomic diamagnetic shielding. This shielded acetoxy group might be either at C-7 of ring D which is close to the unsaturated system of chalcone or at C-4 of ring B which might be under the influence of ring current of ring E.

The assignment of 3(8"-) flavonyl flavanone structure to BGH-III was further supported by mass spectral studies of its methyl ether (LXXVII) (Scheme V). The fragmentation pattern was in agreement with that of BGH-II heptamethyl ether.

On the basis of above evidences BGH-III and its acetate were assigned the structures 4',4"',5,5",7,7"-hexahydroxy-3(8"-) flavonyl flavanone (LXXIII) and 2',4,4',4"',5",6',7"-heptaacetyl-α(8"-) flavonyl chalcone (LXXVIII).


OAc
OAc
AcO
H
OAc
AcO
O
AcO
O
AcO
O
Acetylation
(LXXVIII)
OH
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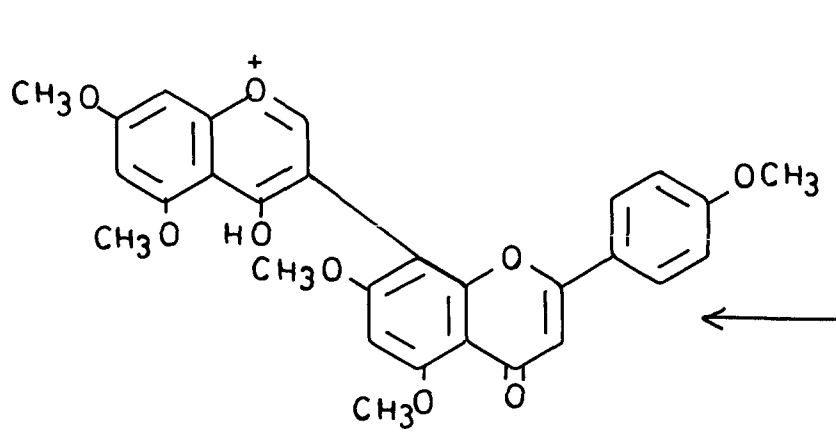
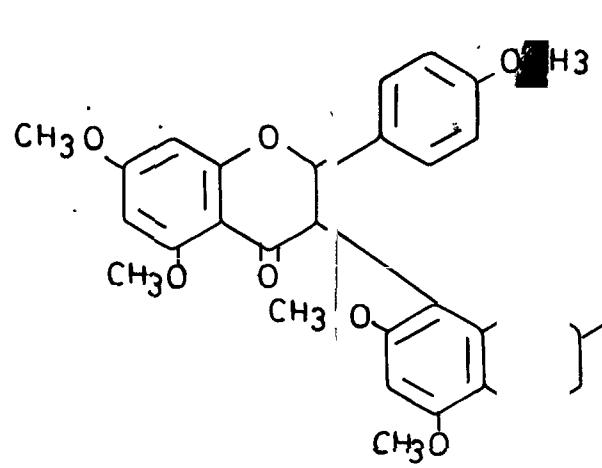
OH
OH
O
HO
O
OH
O
(LXXIII)
Methylation
OCH3
CH3O
O
OCH3
CH3O
O
CH3O
O
CH3O
O
(LXXVII)

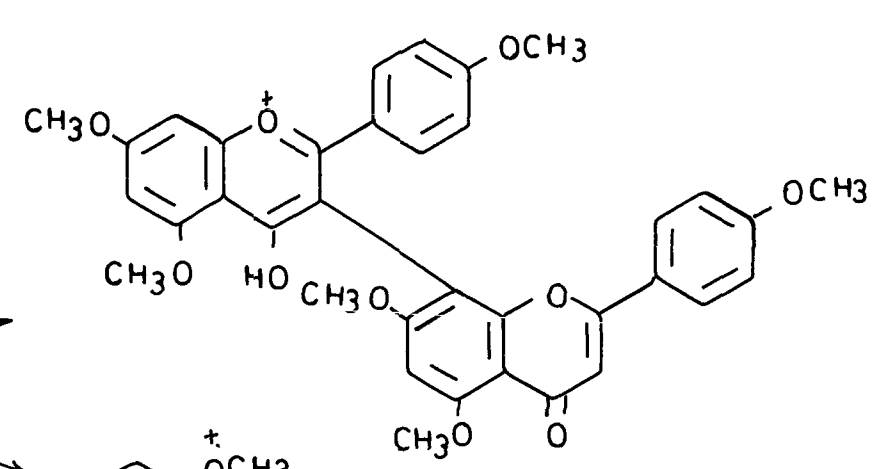
SCHEME - V



m/e 517 (5)



M+, m/e 624 (38)



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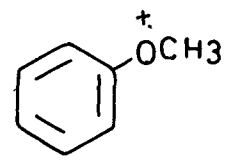

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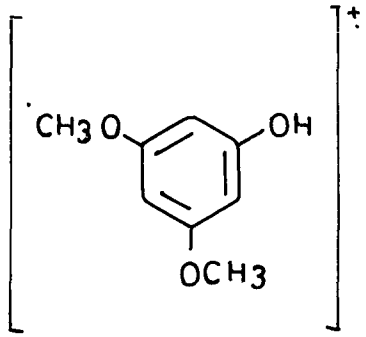
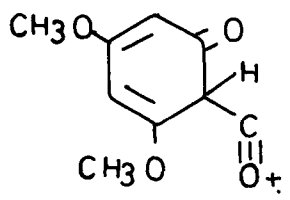
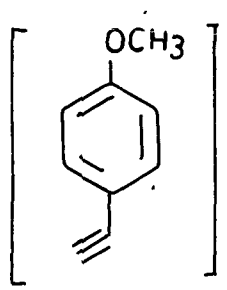


m/e 154 (36)



m/e 18 (84)



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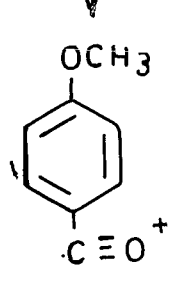
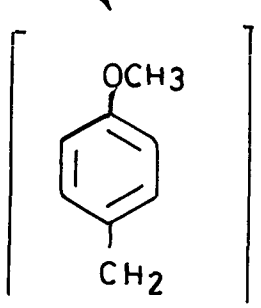
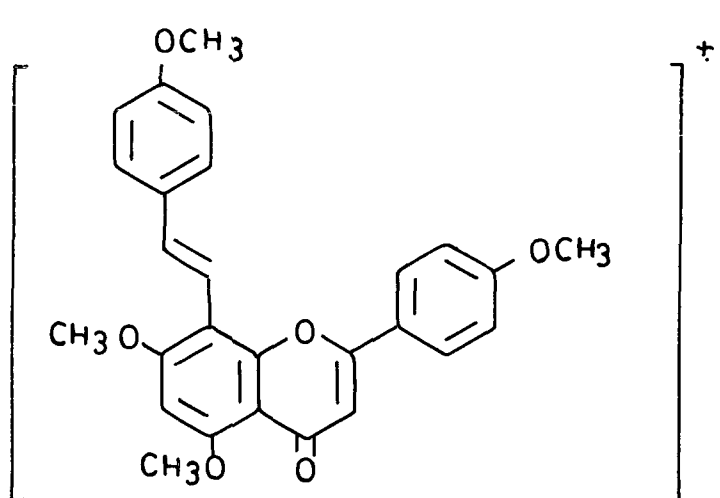


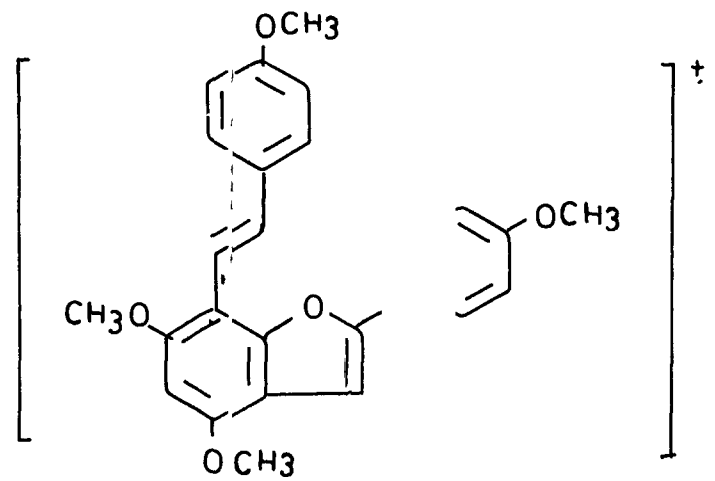
m/e 13 2)



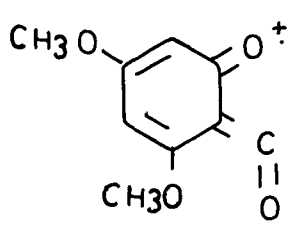
m/e 121 (100)



m/e 444 (50)



m/e 415(



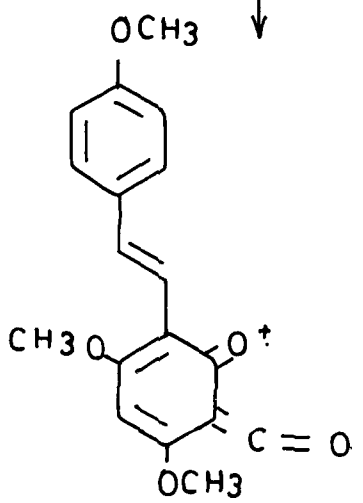
m/e 180 (15)

m/e 152

-CO

-Me

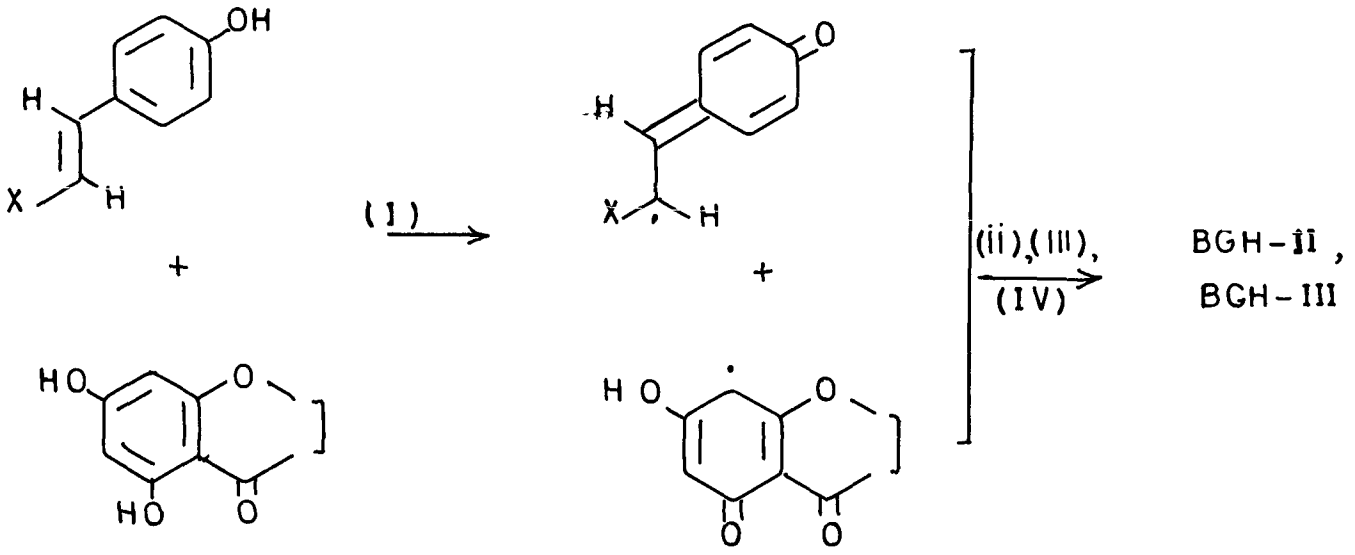
m/e 137



m/e 311 (40)

The phenolic extractives of well powdered deffatted bark of Garcinia livingstonii on solvent fractionation and repeated preparative thin-layer chromatography yielded two components labelled as BGB-II and BGB-III (R_F 0.02 and 0.211 respectively). These components were found to be identical with BGH-II and BGH-III by a comparison of m.ps. mixed m.ps. and R_F values. Their NMR spectra were also identical in all respects with those of BGH-II and BGH-III including solvent dependent methoxy shifts.

It is interesting to note that heartwood and bark of Garcinia species (Guttifereae) which have been investigated so far produce biflavonyls incorporating reduced heterocyclic system(s)[7,9,10]. The two monomeric halves linked only through 3-8" may have the same as well as different oxygenation pattern. If it is assumed that the flavanone formation occurs from a polyacetate and a C_6-C_3 unit then the 3-8" linkage may be formed by oxidative coupling. By analogy with the formation of lignans[64], a hydroxy cinnamic acid moiety (either as a cinnamic acid or a chalcone or a flavone derivative) from flavanone unit may couple with the phloroglucinol residue of another flavone unit or its precursor. The postulated biogenetic pathway is shown below:



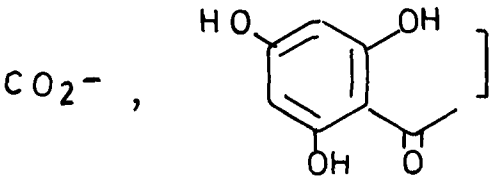
(i) hydrogen abstraction

(ii) radical coupling

(iii) enolization

(iv) cyclization of chalcone

X = possible ligands in flavanone biogenesis.



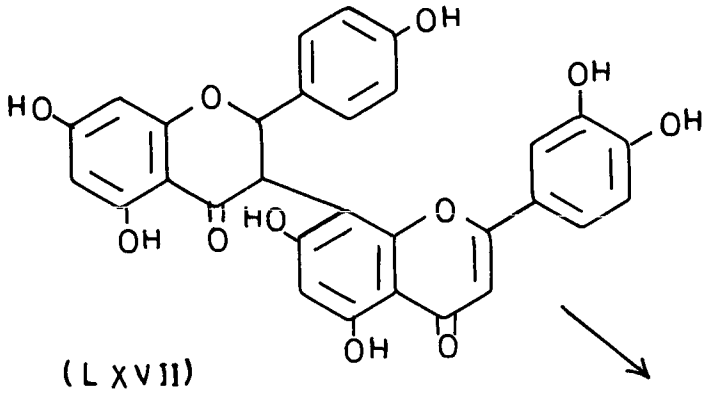
The observation with regard to the existence of biflavonyls derived from two flavone-flavone units (higher

oxidation level) in leaves and those derived from flavanone-flavone units (at lower oxidation level) in heartwood and bark of G. livingstonii agrees well with the earlier suggestions that "there is a tendency for the state of oxidation of flavonoids to increase as the upper extremities of the plants are reached[87]".

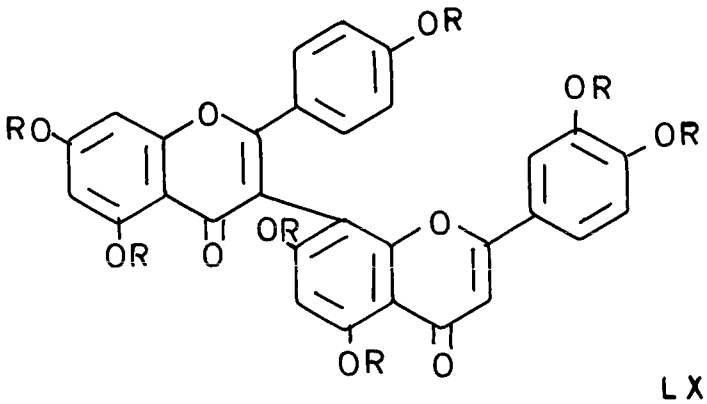
SYNTHESIS OF A NEW SERIES OF BIFLAVONES

Dehydrogenation of flavanones is of importance, as it allows a new flavanone to be rapidly identified by reference to numerous authenticated flavones and, in addition, constitutes a synthesis of flavones which is particularly valuable when other kinds of intermediates are not readily accessible. An important advance in dehydrogenation technique was made when Seshadri et al[88] found that a combination of iodine and sodium acetate in acetic acid dehydrogenates flavanones smoothly. In the present discussion are described the conversions of BGH-II and BGH-III to the corresponding biflavones. Chromatographic and spectral evidences in support of the assigned structures are presented.

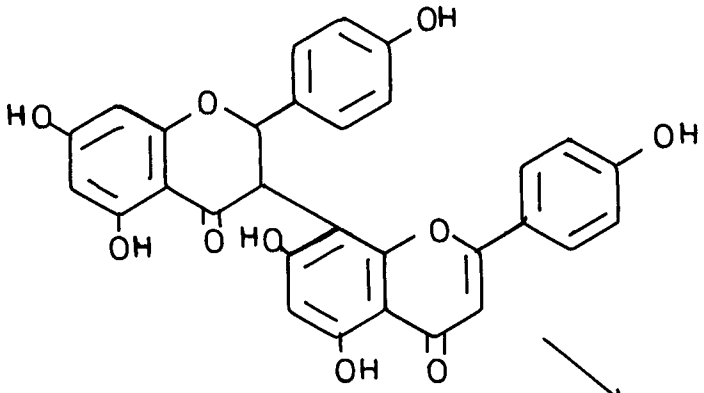
BGH-II (LXVII) (R_F 0.023) and BGH-III (LXXIII) (R_F 0.21) were isolated from Garicinia livingstonii. Both the constituents on treatment with iodine-sodium acetate in acetic acid gave WGH-II (LXXIX) (R_F 0.001) and WGH-III (LXXXI)(R_F 0.16) respectively. The transformation of saturated pyrone ring C to unsaturated one was indicated by the decrease in R_F values. (LXXIX) and (LXXXI) on methylation with methyl iodide-potassium carbonate in acetone gave WGH-II heptamethyl ether (LXXX) and WGH-III hexamethyl ether (LXXXII) respectively. NMR spectra of



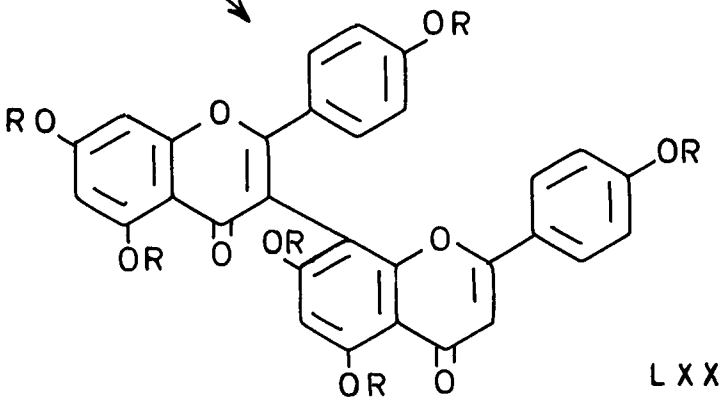
(LXVII)



LXXIX R=H
LXXX R=Me



(LXXIII)



LXXXI R=H
LXXXII R=Me

complete methyl ethers of WGH-II and WGH-III were identical with those of complete methyl ethers of BGH-II and BGH-III except that the aliphatic protons of ring C were absent in the former cases. Results of NMR studies of(LXXX)and (LXXXII)are recorded in table XIV.

T A B L E - XIV*

Proton	LXXX	LXXXII
H-2',6'	2.68 (d) J = 9 c/s	2.67 (d) J = 9 c/s
H-3',5'	3.20 (d) J = 9 ,,	3.32 (d) J = 9 ,,
H-6	3.44 (d) J = 3 ,,	3.42 (d) J = 3 ,,
H-8	3.62 (d) J = 3 ,,	3.60 (d) J = 3 ,,
H-6"	3.49 (s) -	3.52 (s) -
H-3"	3.61 (s) -	3.60 (s) -
H-2"'	2.94 (d) J = 2 c/s	
H-2",6"'		2.51 (d) J = 8 c/s
H-5"'	3.32 (d) J = 10 c/s	
H-3"',5"'		3.32 (d) J = 8 c/s
H-6"'	2.74 (q) J_1 = 10 c/s J_2 = 2 ,,	
OMe	6.00-6.56(21 protons)	6.00-6.29 (18 protons)

s = singlet, d = doublet, q = quartet.

* spectra run at 100 M c/s in $CDCl_3$ (TMS as internal standard = τ 10.00).

The proton assignment has been done by using double irradiation technique. A striking feature in the NMR spectrum of(LXXX)was the appearance of a methoxy group at an exceptionally high position (τ 6.56) suggestive of its being entirely solvated. Examination of the molecular model of(LXXX)was revealing in that there are, infact, certain conformations in which the particular methoxy group could lie above the plane of benzene ring of another flavone unit, thus rendering it unique. Further on change of solvent from $CDCl_3$ to C_6D_6 all the methoxy groups were expected to move upfield more than 30 c/s as each methoxyl had a proton ortho to it[6,32,40,41]. The methoxy group in question (at τ 6.56) however, did not move at all (Fig.VI) thus supporting the above postulate. This does mean, of course, that when we are dealing with a methoxy group in

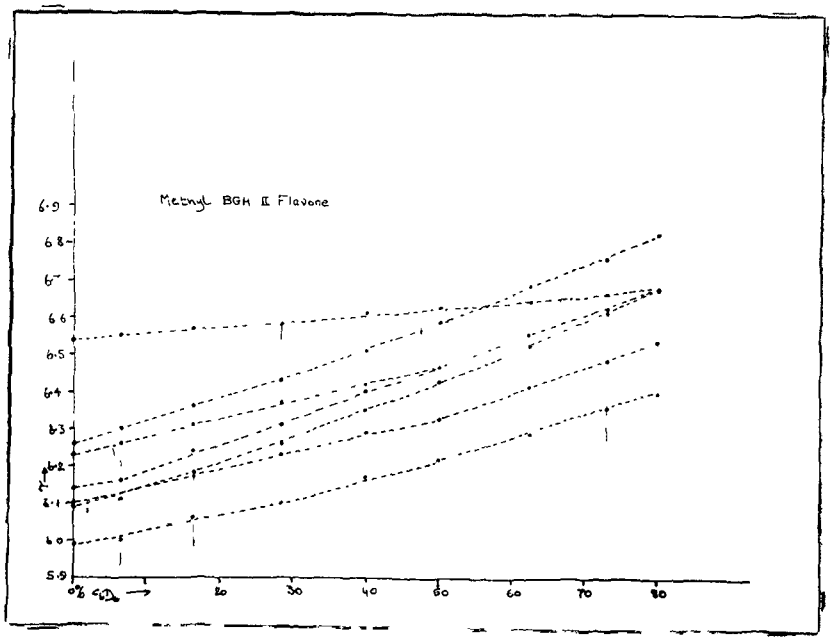


Fig. VI

an unusual position as regards an aromatic methoxy group, the method of solvent induced shifts must be used with greatest caution.

On going from $CDCl_3$ to C_6D_6 all the methoxyl resonances (τ 6.00 - 6.29) in (LXXXII) as expected moved upfield by more than 30 c/s (Fig. VII).

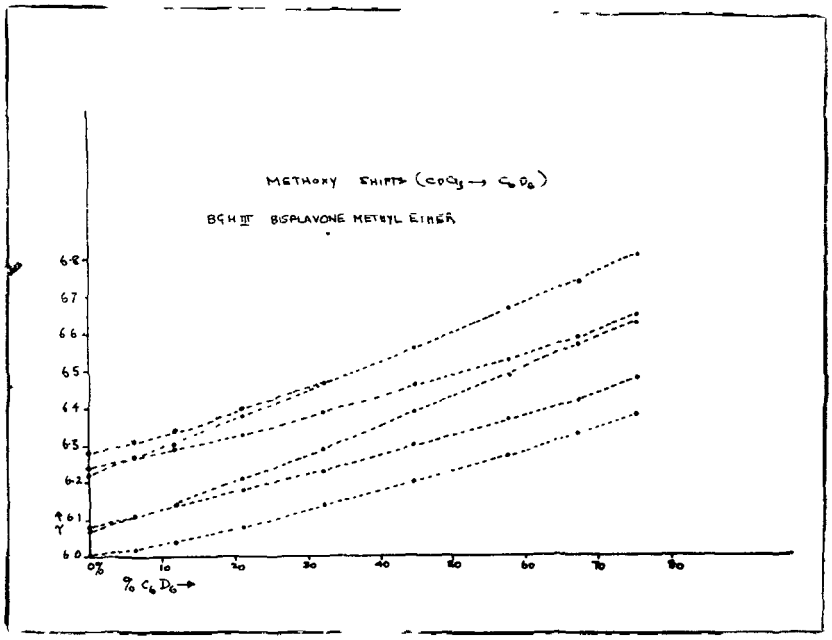


Fig. VII

On the basis of the above discussion WGH-II and WGH-III have been assigned the structures 3"',4',4"',5,5", 7,7"-heptahydroxy-3,8"-biflavone (LXXIX) and 4',4"',5,5", 7,7"-hexahydroxy-3,8"-biflavone (LXXXI) respectively.

THIN-LAYER CHROMATOGRAPHY OF BIFLAVONYLS ON SILICA GEL STRUCTURE-CHROMATOGRAPHIC BEHAVIOUR CORRELATIONS

Earlier methods of detecting flavonoid pigments in plant extracts were based on simple colour tests, many of which were of doubtful validity, when used on crude extracts. Chromatographic methods have the advantages that they separate the flavonoids and provide an accurate and rapid means of provisional identification based on R_F values and colour reactions. Further these methods can be applied to as little as 1 mg of unknown substances and many pigments have been identified without being isolated on a macro scale. Lastly the diversity of R_F values that can be obtained for each compound run in a variety of solvent mixtures provides a most useful aid to characterization.

Paper chromatography was first used for the separation of flavonoid pigments by Bate-Smith (1948)[89]. Since then it has been used increasingly in the study of these compounds [16-19] which are idealy suited to this particular technique. The flavonoids have just the right range of solubility characteristics for ease of separation and most of them possess characteristic colours on paper in visible or ultraviolet light.

Some of the most frequently used solvent systems for the separation of monoflavonoids are listed below:

1.	n-Butanol:acetic acid:water	4:1:5 v/v (organic layer)
2.	n-Butanol:acetic acid:water	6:1:2 v/v (miscible)
3.	n-Butanol:27% acetic acid	1:1
4.	n-Propanol:acetic acid:water	1:1:1
5.	Acetic acid:water	1:9, 2:8, 3:7, 4:6, 5:5 etc.
6.	Acetic acid:Conc. HCl:water (Forestal)	30:3:10
7.	m-Cresol:acetic acid:water	50:2:48
8.	Ethyl acetate:acetic acid:water	50:2:50
9.	Benzene:acetic acid:water	125:72:3
10.	Chloroform saturated with water	
11.	Ethyl acetate saturated with water	

The exact proportions of solvents used in a mixture are not usually critical and indeed may be varied on occasions to give improved separations.

Sawada[83] and Hasegawa[90], in their studies on taxonomic distribution of biflavonyls among gymnosperms have detected most of the biflavonyls by paper chromatography. Di Modica et al[20], during isolation of biflavonyls from Taxus baccata, observed that "with the classical solvent systems, the separations of biflavonyls, using both ascending and descending techniques on different chromatographic papers have not been satisfactory". The reasons suggested were:

(a) high migration speed of examined biflavones.

(b) R_F values of test samples were too close together (table XV).

T A B L E - XV

Compound	AcOH:H_2O 2:3	Isopropanol: water 3:2	EtOAc satd. with water	$CHCl_3$ satd. with water
Quercetin	0.28	0.74	0.82	
Ginkgetin	0.94	0.96	0.95	0.76
Sciadopitysin	0.81	0.96	0.95	0.77
Flavone 310 (from Taxus baccata)	0.80	0.95	0.95	0.71

Attempts of Baker et al[3] to achieve quantitative separation of biflavones from various plant extracts by paper chromatography (Whatman No. 3) also proved futile. The following solvent mixtures were tried:

(a) n-Butanol:15N aqueous ammonia:5N aqueous ammonium carbonate (1:1:1 v/v, top layer).

(b) Ethanol:15N aqueous ammonia:water (90:5:5 v/v)

Thin-layer chromatography, the technique redeveloped by Stahl[91] in later fiftees as a tool for quantitative

separations, has many superiorities, such as speed, sensitivity, and efficiency of separation when compared to paper chromatography for the analysis of many types of compounds. Its development in past few years has provided the organic chemist with a new method of analysis. The method has not extensively been used as yet for the identification of phenolic compounds although a variety of simple phenols such as hydroxy benzoic and cinnamic acids[92,93], coumarins[95], anthocyanadins[93,94], flavones[95], and simple glycosides have been separated on thin layers of silica gel, Kieselguhr and polyamide.

There have been some isolated reports as to the use of thin-layer chromatography for separation of biflavones from plant extracts[7,21]. Kawano et al[21] using this technique has revised many findings of Sawada[83] on the occurrence of biflavones in twelve gymnosperms. A review of the literature revealed that no comprehensive study on the use of thin-layer chromatography for detection, identification and quantitative separation of biflavones had been undertaken so far probably because of the availability of a few biflavonyls belonging to only two series.

In the present discussion is described a systematic study on thin-layer chromatography of 38 biflavonyls and their derivatives, representing all the series known todate, using five different solvent systems.

The biflavonyls were either isolated from natural sources or synthesized in laboratory (details given in experimental part).

The following 5 mixtures were studied as developing solvent systems:

1. Benzene:pyridine:formic acid (BPF) 36:9:5[96]
2. Toluene:ethyl formate:formic acid (TEFF) 5:4:1[95]
3. Toluene:pyridine:acetic acid (TPA) 10:1:1[47]
4. Benzene:ethyl acetate:acetic acid(BEAA) 8:5:2
5. Benzene:pyridine:ethyl formate:dioxan (BPEFD) 5:1:2:2

All the spots were located in UV light but the parent biflavonyls and their partial methyl ethers were also revealed using $FeCl_3$-EtOH and diazotized sulphanilic acid[97] as chromogenic reagents. R_F values of biflavonyls, their partial and fully methylated derivatives (Table XVI) were obtained under closely comparable conditions and were calculated to an average of three values.

Both chromogenic reagents, $FeCl_3$-EtOH and diazotized sulphanilic acid were found useful in revealing the presence of biflavonyls and their partial methyl ethers. Diazotized sulphanilic acid, however, had the additional advantage of giving an approximate idea about the extent of methylation as the colour changed from dark brown to yellow with increasing

T A B L E - XVI

Compound	BPF	TEFF	TPA	BPEFD	BPAA	Colour (solvent BPF)
Apigenin (XXXIII)	0.518					
Amentoflavone (Ia)	0.173	0.327	0.067	0.433	0.273	Dark brown[a]
Sotetsuflavone (Ib)	0.373	0.527	0.160	0.520	0.480	Light brown[a]
Bilobtin (Ic)	0.373	0.526	0.160	0.520	0.480	,, ,,
Podocarpusflavone A (Id)	0.373	0.527	0.160	0.520	0.480	,, ,,
Isoginkgetin (Ig)	0.540	0.570	0.340	0.713	0.700	Orange brown[a]
Ginkgetin (Ih)	0.540	0.570	0.340	0.713	0.700	,, ,,
Kayaflavone (Ii)	0.613	0.607	0.553	0.820	0.793	Orange[a]
Sciadopitysin (Ij)	0.613	0.607	0.553	0.820	0.793	,,
Tetra-O-methyl amento-flavone (Il)	0.763	0.663	0.660	0.907	0.88	Yellow[a]
Cupressuflavone (IIa)	0.163	0.300	0.067	0.360	0.287	Dark brown[a]
7-O-Methyl cupressuflavone (IIb)	0.360	0.520	0.160	0.510	0.476	Light brown[a]
7,7"-Di-O-methyl cupressuflavone (IIc)	0.500	0.573	0.340	0.607	0.605	Orange brown[a]

TABLE - XVI (Contd.)

Compound	BPF	TEFF	TPA	BPEFD	BEAA	Colour (solvent BPF)
4',7,7"-Tri-O-methyl cupressuflavone	0.607	0.600	0.552	0.820	0.790	Orange[a]
4',4"',7,7"-Tetra-O-methyl cupressuflavone (IId)	0.76	0.630	0.620	0.897	0.850	Orange[a]
Agathisflavone (III)	0.157	0.233	0.040	0.353	0.267	Dark brown[a]
Agathisflavone A(IIIa)	0.274	0.437	0.100	0.407	0.357	Light brown[a]
Agathisflavone B(IIIb)	0.427	0.570	0.233	0.607	0.593	Orange brown[a]
7,7"-Di-O-methyl agathisflavone (IIIc)	0.427	0.570	0.233	0.607	0.593	,, ,,
BGH-II (LXVII)	0.023	0.187	0.017	0.254	0.273	Dark brown[a]
BGH-III (LXXIII)	0.211	0.36	0.080	0.481	0.384	,, ,,
WGH-II (LXXIX)	0.001	0.15	0.001	0.23	0.161	,, ,,
WGH-III (LXXXI)	0.16	0.30	0.003	0.39	0.280	,, ,,
GB-1 (IVb)	0.139	0.373	0.043	0.265	0.380	Reddish brown[a]
GB-2 (IVd)	0.061	0.187	0.020	0.227	0.287	,, ,,

TABLE - XVI (Contd.)

Compound	BPF	TEFF	TPA	BPEFD	BEAA	Colour (solvent BPF)
Hinokiflavone (VIIa)	0.320	0.373	0.133	0.474	0.353	Brown[a]
Cryptomerin A (VIIa)	0.573	0.487	0.327	0.61	0.73	Light brown[a]
Isocryptomerin (VIIb)	0.573	0.487	0.327	0.61	0.73	,, ,,
Cryptomerin B (VIIe)	0.673	0.553	0.487	0.692	0.80	Orange brown[a]
Amentoflavone (3'-8") hexamethyl ether (XXVII)	0.404	0.353	0.087	0.085	0.06	Bright yellow[b]
Amentoflavone (3'-6") hexamethyl ether (XXVIII)	0.497	0.517	0.267	0.415	0.273	Blue[b]
Cupressuflavone (8-8") hexamethyl ether (XXV)	0.428	0.467	0.173	0.170	0.13	Orange[b]
Agathisflavone (6-8") hexamethyl ether (XXIX)	0.449	0.477	0.200	0.315	0.197	Bright yellow with light green tinge[b]
Hinokiflavone (4'-O-8") pentamethyl ether (XXX)	0.438	0.453	0.110	0.130	0.08	Dull yellow[b]
Hinokiflavone (4'-O-6") pentamethyl ether (XXXI)	0.522	0.527	0.283	0.546	0.13	Blue[b]

TABLE - XVI (Contd.)

Compound	BPF	TEFF	TPA	BPEFD	BEAA	Colour (solvent BPF)
BGH-II heptamethyl ether(LXXII)	0.395	0.340	0.08	0.20	0.10	Yellow with greenish tinge[b]
BGH-III hexamethyl ether (LXXVIII)	0.420	0.370	0.140	0.26	0.164	Yellow[b]
WGH-II heptamethyl ether (LXXX)	0.370	0.300	0.041	0.141	0.073	Orange[b]
WGH-III hexamethyl ether (LXXXII)	0.401	0.340	0.090	0.192	0.112	,,

a Diazotized sulphanilic acid as spray reagent,

b in UV light.

methylation. Examination of the fully methylated biflavones (BPF) in UV light provided a fairly good diagnostic test for the identification of different series of biflavones (Table XVI). The spots were compact and the differences in R_F values of all members of a series in BPF were so marked (Fig. VIII, chromatogram 1) that it became the developing system of choice not only for satisfactory identification but also for quantitative purposes. This solvent system was found to work equally well for biflavonyls with reduced heterocyclic rings where most of the systems proved unsuccessful. In BPEFD, although the spots were compact the R_F value differences between the parent compound and partial methyl ethers in a series were small (Fig. VIII, chromatogram 4). The system, however, proved to be most satisfactory in the quantitative separation of isomeric mixtures of fully methylated biflavonyls. BEAA, TPA and TEFF (the last one most widely used by Kawano et al[21,98,99]) were found to be of some value for qualitative work (Fig. VIII, chromatograms 2,3,5) but of no value at all in quantitative separations. The spots were either too elongated or too closely spaced, and in some cases travelled with the solvent front. BEAA and TPA were found unsatisfactory for morelloflavone, WGH-II, GB-series, and fully methylated biflavonyls (Table XVI).

All the biflavonyls except those belonging to GB and BGH series and their derivatives are derived from apigenin

units with a C-C or C-O-C interflavonyl linkage. It is well known that if there are several substituents in the same molecule, the effect of each substituent on the adsorption affinity is approximately additive[100]. A comparison of R_F values of biflavonyls [amentoflavone (Ia), cupressuflavone (IIa), agathisflavone (III) and WGH-III (LXXXI)] consisting of two apigenin units with the R_F value of apigenin itself indicates that the aforesaid generalization is far from being even very approximate. Amentoflavone (Ia), cupressuflavone (IIa), agathisflavone (III) and WGH-III (LXXXII) having an equal number of phenolic hydroxyls would be expected to show the same R_F values. It has actually been found that their R_F values are so close as to defy their identification and separation in cases where they occur together in the same plant.[82] The small differences in R_F values (BPF) of amentoflavone (0.173); cupressuflavone (0.167), agathisflavone (0.163) and WGH-III (0.16) may, however, be explained by their relative departure from planarity with subsequent variations in the magnitude of conjugative effects.

In the same series, a monomethyl ether shows an R_F value higher than that of the parent compound, a dimethyl higher than a monomethyl ether, a trimethyl higher than a dimethyl ether etc. For example: see the R_F's of Ia, Ib, Ig and Ii. The trend is in line with the observation that the increase in R_F values parallels the increase in methyla-

tion[18,19,101,102]. Isomeric methyl ethers of the same series (amentoflavone) such as sotetsuflavone (Ib), bilobtin (Ic), podocarpusflavone A (Id) (monomethyl ethers), ginkgetin (Ih), isoginkgetin (Ig) and podocarpusflavone B (dimethyl ethers); kayaflavone (Ii) and sciadopitysin (Ij) (trimethyl ethers), show the same R_F values (Table XVI) in accordance with the observation that the position of substituent is of secondary importance[103].

Hinokiflavone (VIIa) a biflavone of biphenyl ether pattern shows an R_F of 0.32 (BPF). This is much higher than the R_F value of the four biphenyl type biflavones discussed earlier. Hinokiflavone with five free phenolic hydroxyls and the sixth involved in an ether type interflavonyl linkage may be expected to behave, with respect to adsorption affinity, like a monomethyl ether of biphenyl type biflavones. Similarly the monomethyl ethers of hinokiflavone compare with the dimethyl ethers of amentoflavone and cupressuflavone. The validity of this argument is supported by the observation that such mixtures when encountered in these laboratories, could not be separated by TLC[82].

The adsorption affinity differences widen with the increasing methylation so much so that the fully methylated biflavones including various modes of interflavonyl linkages were found to show sizable differences in R_F values. The

observation has been exploited extensively and successfully in these laboratories to bring about the separation, in BPF and BPEFD, of pure samples from fully methylated mixtures of amentoflavone (XXVII) (R_F 0.40), cupressuflavone (XXV) (R_F 0.43), agathisflavone (XXIX) (R_F 0.45) and hinokiflavone (XXXI) (R_F 0.52), although it was not possible to separate them at the partial methyl ether stage. The characteristic fluorescence of these derivatives in UV light (BPF) was also found to be of some help in their identification. BPF and BPEFD have, thus, been established as excellent developing systems for the quantitative separation of fully methylated biflavones.

The increasing trend in R_F values of hexamethyl ethers of amentoflavone (XXVII), WGH-III (LXXXII), cupressuflavone (XXV) and agathisflavone (XXIX) respectively may be interpreted on the magnitude of their departure from planarity as a result of steric interactions. Examination of space models reveals that such interactions are greater in the case of agathisflavone hexamethyl ether than in amentoflavone hexamethyl ether resulting in subsequent deviation from planarity. This is translated as a decrease in the adsorption affinity and the corresponding increase in the R_F value of agathisflavone hexamethyl ether. Similar arguments may be used to explain why fully methylated biflavonyls involving C-6 in the interflavonyl linkage show higher R_F values (BPF) than those involving C-8. This is shown in table XVII.

T A B L E - XVII

Biflavone methyl ether	C-8	C-6
Hinokiflavone	0.44	0.52
Amentoflavone	0.40	0.50
Cupressuflavone	0.43	0.45 (Agathisflavone)

The saturation of the double bond in the heterocyclic ring of a biflavone nucleus causes an increase in the R_F values (Table XVIII). The increase in R_F values of biflavonyls having saturated pyrone ring(s) in comparison to those having unsaturated heterocycle(s), having the same number of hydroxyls/methoxyls, is attributable to greater mobility as a result of more nonplaner configuration of the former[18,19,101,102].

T A B L E - XVIII

Biflavonyls with saturated ring C	R_F value	Biflavonyls with unsaturated ring C	R_F value
BGH-II (LXVII)	0.023	WGH-II (LXXIX)	0.001
BGH-III (LXXIII)	0.211	WGH-III (LXXXI)	0.160
BGH-II heptamethyl ether (LXXII)	0.395	WGH-II heptamethyl ether (LXXX)	0.370
BGH-III hexamethyl ether (LXXVIII)	0.420	WGH-III hexamethyl ether (LXXXII)	0.401
GB-1 (IVb)	0.139	BGH-II (LXVII)	0.023

The lower R_F value of BGH-II (LXVII) and GB-2 (IVd) as compared to BGH-III (LXXIII) and GB-1 (IVb) respectively, both with identical configuration may be interpreted as due to greater phenolic contents of the former.

Although the detailed studies on structure - chromatographic behaviour correlations have made it possible to detect and provisionally identify biflavonyls, some caution must be excercised in drawing final conclusions. Complete dependence on literature R_F values without running a chromatogram of plant extracts along with authentic samples may lead to oversight of closely related new constituents. It is, therefore, desirable to isolate the constituents in pure state and make a direct comparison with the authentic samples. It is also noteworthy that the chromatographically homogenous components may and may not be pure compounds.

C O N C L U S I O N S

A. Biflavonyls from Guttifereae:

(i) The phenolic extractives of the leaves, heartwood and bark of Garcinia livingstonii have yielded four biflavonyls, one of them being reported for the first time.

(ii) The structure of each of the component has been elucidated by chromatographic, UV, IR, mass and NMR studies including double irradiation technique and solvent dependent methoxyl resonances.

(iii) The following structure have been assigned:

(a) 4',4"',5,5",7,7"-Hexahydroxy-3',8"-biflavone (amentoflavone, WG-1).

(b) 4"'-O-Methyl amentoflavone (podocarpusflavone A, WG-2).

(c) 3"',4',4"',5,5",7,7"-Heptahydroxy-3(8"-) flavonyl flavanone (BGH-II).

(d) 4',4"',5,5",7,7"-Hexahydroxy-3(8"-)flavonyl flavanone (BGH-III; new biflavonyl)

The heartwood and bark are found to contain the same constituents.

(iv) The presence of amentoflavone and podocarpusflavone A,

flavone-flavone type dimers in leaf extracts of Garcinia species is being reported for the first time.

(v) An unusual observation of the transformation of (c) and (d) to the corresponding chalcones was made during acetylation using acetic anhydride-pyridine.

B. Synthesis of a new series of biflavones:

(i) Two members belonging to a new series of biflavones have been synthesized. NMR studies including double irradiation technique and solvent induced shifts of methoxy resonances have been used for structure elucidation. An anamoly in the method of methoxy proton shifts is pointed out.

(ii) The assigned structures are:

(a) 3"',4',4"',5,5",7,7"-Heptahydroxy-3,8"-biflavone (WGH-II).

(b) 4',4"',5,5",7,7"-Hexahydroxy-3,8"-biflavone (WGH-III).

C. Thin-layer chromatography of biflavonyls on silica gel. Structure-chromatographic behaviour correlations:

(i) The chromatographic behaviour of biflavonyls including partially and fully methylated ethers, has been examined in five solvents.

(ii) Benzene:pyridine:formic acid (36:9:5) has been found to be the most satisfactory developing system both for identification and separation of biflavonyls and their methyl ethers.

(iii) Benzene:pyridine:ethyl formate:dioxan (5:1:2:2) has been claimed best for fully methylated biflavonyls.

(iv) Relative R_F values coupled with variations in the shades developed by spraying with diazotized sulphanilic acid have been used for ascertaining approximately, the extent of the methylation in partial methyl ethers of the same series.

(v) The characteristic shades of fully methylated biflavonyls in UV light have been found to provide a means for their quick and satisfactory identification.

(vi) An attempt has been made to correlate the structure of biflavonyls and their methyl ethers with their chromatographic behaviour. Isomeric pairs of fully methylated biflavonyls involving different interflavonyl linkages, such as hinokiflavone (4'-O-8", 4'-O-6") amentoflavone (3'-8", 3'-6"), cupressuflavone (8-8") and agathisflavone (6-8") have easily been distinguished and oriented.

E X P E R I M E N T A L

BIFLAVONYLS FROM GUTTIFEREAE

Biflavones from the leaves of Garcinia livingstonii:

Dried and powdered leaves of G. livingstonii (2.5 Kg) were refluxed with petroleum ether (40-60°, 3 x 8 liters, for 8 hr each). The combined extracts were concentrated under reduced pressure. An oily green residue left behind showed no flavonoid colour tests and was rejected.

The petrol treated leaves were completely dried and exhausted with boiling acetone till the extract was almost colourless. The combined acetone extracts were concentrated under diminished pressure. A highly viscous dark green concentrate was left behind. This was refluxed successively with petroleum ether (40-60°), benzene and chloroform to remove non-flavonoidic constituents. The residue was then treated with boiling water. The insoluble mass was dissolved in alcohol and dried under reduced pressure. The green solid thus obtained gave flavonoid colour reactions.

Purification of biflavone mixture-Column chromatography:

A well stirred suspension of magnesium silicate (Woelm; 120 gm, 400 ml of dry petroleum ether, 40-60°) was poured into a column (150 cm long and 50 mm in diameter).

When the adsorbent was well settled, excess of petroleum-ether was allowed to flow out. Crude mixture of biflavones (2.5 gm) dissolved in dry acetone (40 ml) was introduced into the column. After the starting zone had been formed, a circular filter paper was placed at the top of the adsorbent. The column was developed with organic solvents in the increasing order of polarity. Results are given in table XIX.

T A B L E - XIX

Solvent	Nature of product
(i) Petroleum-ether (40-60°)	Greenish gummy mass
(ii) Benzene	Greenish waxy product
(iii) Chloroform	Green non-flavonoidic solid
(iv) Ethyl acetate	Yellow solid (425 mg)
(v) Acetone	Yellow solid (580 mg)
(vi) Ethyl acetate (saturated with water)	Yellow solid (600 mg)
(vii) Alcohol	Brownish gummy mass

Products eluted with ethyl acetate, acetone and ethyl acetate (saturated with water) gave usual flavonoidic colour tests.

Colour Tests:

1.	Magnesium + hydrochloric acid	Orange
2.	Alcoholic ferric chloride	Greenish brown
3.	Zinc + hydrochloric acid	Red
4.	Sodium-amalgam + hydrochloric acid	Pinkish violet

Separation of biflavones-Preparative thin-layer chromatography:

Using thin-layer spreader (Desaga, Hiedelberg) glass plates (20 x 20 cm) were coated with well stirred suspension of silica gel G (E. Merck, 50 gm, 95 ml water) to give a layer approximately 0.5 mm in thickness. After drying for two hr at room temperature, the plates were activated at 110-120^{o} for 1 hr and preserved in a desiccator until required.

Thin-layer chromatographic examination of products obtained with ethyl acetate, acetone and ethyl acetate (saturated with water) in seven solvent systems, listed below, indicated the presence of two components in each case. The three fractions (1.6 g) were combined and subjected to preparative thin-layer chromatographic separation.

Solvent systems used:

(a)	Benzene:pyridine:formic acid	36:9:5
(b)	Toluene:ethylformate:formic acid	5:4:1

(c)	Toluene:pyridine:acetic acid	10:1:1
(d)	Benzene:ethyl acetate:acetic acid	8:5:2
(e)	Benzene:ethylformate:pyridine: dioxan	5:2:1:2
(f)	Benzene:acetone	7:3
(g)	Benzene:acetone	1:1

In solvent system (a) the spots were compact and differences in R_F values were quite marked. This solvent system was,therefore,used for all the subsequent separations of biflavonyls.

Solution of pure biflavonoidic mixture in pyridine (4%) was applied to plates with the help of mechanical applicator (Desaga, Hiedelberg) 2 cm from the lower edge of the plates. The plates mounted on stainless steel frame were placed in a Desaga glass chamber 45 x 22 x 25 cm containing 500 ml of the developing solvent system (Benzene: pyridine:formic acid, 36:9:5). When the solvent front had travelled 15 cm from the starting line the development was interrupted and the plates were dried at room temperature. The position of bands was marked in UV light. The marked pigment zones were scraped with the help of spatula and eluted with dry acetone in separate columns. The eluent in each case was distilled off to give an oily liquid which on addition of water yielded yellow precipitate. It was filtered, washed with water and dried. Homogeneity of the

pigments was checked again by TLC using all the seven solvents systems already listed.

4',4"',5,5",7,7"-Hexahydroxy-3',8"-biflavone (amentoflavone) (WG-1):

Crystallized from methanol as pale yellow rods (430 mg), m.p. 255-56°, R_F 0.173, $[\alpha]_D^{25}$ + 9 (pyridine-ethanol, 1 mg/ml), λ_{max}^{EtOH} 272, 335 nm, ν_{max}^{KBr} 3027, 1648, 1604, 1568, 1499, 1425, 1358, 1285, 1237, 1172, 1160, 1107, 1047, 1027, 996, 984, 946, 910, 835, 767, 752 and 732 cm^{-1}, [Found: C, 60.76; H, 4.18, Calcd. for $C_{30}H_{18}O_{10}\cdot 3H_2O$: C, 60.81; H, 4.08].

4',4"',5,5",7,7"-Hexaacetyl-3',8"-biflavone:

Biflavone (100 mg), pyridine (1.5 ml) and acetic anhydride (1.5 ml) were heated at 95° on a water bath for two hr. The reaction mixture was cooled and poured onto crushed ice. The white solid was filtered, washed, dried and crystallized from chloroform-methanol as colourless needles (85 mg), m.p. 235°, [Found: C, 63.6; H, 3.86, Calcd. for $C_{42}H_{30}O_{16}$: C, 63.8; H, 3.8. Mol. Wt. 790 (mass)].

NMR ($CDCl_3$): values on τ scale:

2.92 (d, J = 9 c/s, 2H, H-3"',5"'); 2.50 (d, J = 9 c/s, 2H, H-2"',6"'); 1.99 (q, J_1 = 9 c/s and J_2 = 3 c/s, 1H, H-6');2.48 (d, J = 9 c/s, 1H, H-5'); 1.94 (d, J = 3 c/s, 1H, H-2'); 3.30, 3.32 (s, 2H, H-3,3"); 2.73 (d, J = 3 c/s, 1H, H-8); 3.31 (d, J = 3 c/s, 1H,H-6); 2.97 (s, 1H, H-6"); 7.50, 7.54 (s, 3H each, OAc -5,5") 7.89, 7.93 (s, 3H each, OAc-7,7"); 6.67, 7.72 (s, 3H each, OAc-4',4"').

4',4"',5,5",7,7"-Hexa-O-methyl-3',8"-biflavone (WG-3):

A mixture of biflavone (100 mg), anhydrous potassium carbonate (2 g) and methyl iodide (1 ml) in dry acetone (100 ml) was refluxed for 10 hr, with further addition of methyl iodide (0.5 ml) and potassium carbonate (1 g), after 4 hr. The mixture was filtered and the residue washed for 6-7 times with hot acetone. The filtrate and washings were combined and evaporated to dryness. The yellow residue was taken up in chloroform (100 ml). The chloroform solution was washed several times with water in a separatory funnel. It was then reduced to 20 ml and purified by preparative thin-layer chromatography. The white solid crystallized from chloroform-methanol (60 mg), m.p. 225°, R_F 0.404, $[\alpha]_D^{25}$ + 28° (pyridine-ethanol, 1 mg/ml), λ_{max}^{EtOH} 267 mm, 368 mm, ν_{max}^{KBr} 3082, 3000, 1648, 1600, 1572, 1505, 1495,

1460, 1420, 1385, 1338, 1298, 1254, 1213, 1175, 1156, 1115, 1066, 1057, 1026, 980, 952, 932, 911, 832, 822, 807, 722 cm^{-1} [Found: C, 69.3; H, 4.8, Calc. for $C_{36}H_{30}O_{16}$: C, 69.45; H, 4.9, Mol. Wt. 622 (mass)].

NMR ($CDCl_3$): Values on τ scale:

3.24 (d, J = 9 c/s, 2H, H-3"',5"'); 2.60 (d, J = 9 c/s, 2H, H-2"', 6"'); 2.10 (q, J_1 = 8 c/s, J_2 = 3 c/s, 1H, H-6'); 2.88 (d, J = 9 c/s, 1H, H-5'); 2.16 (d, J = 3 c/s, 1H, H-2'); 3.42, 3.48 (s, 2H, H-3,3"); 3.52 (d,J = 3 c/s, 1H, H-8); 3.66 (d, J = 3 c/s, 1H, H-6); 3.38 (s, 1H, H-6"); 5.94, 6.08 (s, 3H each, OMe - 5, 5"); 6.12, 6.28 (s, 3H each, OMe - 7,7"); 6.25, 6.27 (s, 3H each, OMe -4',4"').

4"'-Mono-O-methyl-4',5,5",7,7"-pentahydroxy-3',8"-biflavone (podocarpusflavone A, WG-2):

Crystallized from chloroform-methanol as yellow needles (600 mg), m.p. 228-30°, R_F 0.373, $[\alpha]_D^{25}$ - 6 (pyridine-ethanol, 1 mg/ml), λ_{max}^{EtOH} 273 nm, 336 nm, ν_{max}^{KBr} 3030, 1650, 1602, 1554, 1496, 1425, 1351, 1285, 1260, 1235, 1175, 1157, 1107, 1046, 1026, 997, 982, 943, 909, 831, 768, 748 and 735 cm^{-1}, [Found: C, 64.63; H, 3.80, Calc. for $C_{31}H_{20}O_{10} \cdot 1^1/_2 H_2O$: C, 64.25; H, 3.80].

4"'-Mono-O-methyl-4',5,5",7,7"-pentaacetyl-3',8"-biflavone:

A mixture of biflavone (100 mg), pyridine (1 ml) and acetic anhydride (1 ml) was heated on a water bath for 2 hr. It was poured onto crushed ice. The white solid was filtered, washed, dried and crystallized from chloroform-methanol as colourless needles (70 mg), m.p. 235-236°, [Found: C, 64.44; H, 3.97, Calc. for $C_{41}H_{30}O_{15}$: C, 64.56; H, 3.96, Mol. Wt. 762 (mass)].

NMR ($CDCl_3$): values on τ scale:

3.21 (d, J = 9 c/s, 2H, H-3"', 5"'); 2.58 (d, J = 9 c/s, 2H, H-2"', 6"'); 2.00 (q, J_1 = 9 c/s and J_2 = 3 c/s, 1H, H-6'); 2.49 (d, J = 9 c/s, 1H, H-5'); 1.95 (d, J = 3 c/s, 1H, H-2'); 3.40, 3.41 (s, 2H, H-3,3"); 2.75 (d, J = 3 c/s, 1H, H-8); 3.15 (d, J = 3 c/s, 1H, H-6); 3.01, (s, 1H, H-6"); 7.51 (s, 3H each, OAc - 5,5"); 7.90, 7.94 (s, 3H each OAc, 7,7"); 7.68 (s, 3H, OAc - 4'); 6.25 (s, 3H, OMe - 4"').

4',4"',5,5",7,7"-Hexa-O-methyl-3',8"-biflavone (WG-3):

Biflavone (100 mg), anhydrous potassium carbonate (2 g), methyl iodide (1 ml) and dry acetone (100 ml) were refluxed for 12 hr with further addition of potassium carbonate (1 g) and methyl iodide (0.5 ml) after 6 hr.

After usual work up the methyl ether was recrystallized from chloroform-methanol as colourless needles (60 mg), m.p. 225°, R_F 0.404, λ_{max}^{EtOH} 267 nm, 328 nm, ν_{max}^{KBr} 3082, 3000, 1648, 1600, 1572, 1505, 1495, 1460, 1420, 1385, 1338, 1298, 1254, 1213, 1175, 1156, 1066, 1057, 1026, 980, 952, 932, 911, 832, 822, 807, and 722 cm^{-1}, [Found: C, 69.3; H, 4.8, Calc. for $C_{36}H_{30}O_{10}$: C, 69.45; H, 4.9, Mol. Wt. 622 (mass)].

NMR ($CDCl_3$): values on τ scale:

3.24 (d, J,= 9 c/s, 2H, H-3"', 5"'); 2.60 (d, J = 9 c/s, 2H, H-2"', 6"'); 2.10 (q, J_1 = 9 c/s and J_2 = 3 c/s, 1H, H-6'); 2.88 (d, J = 9 c/s, 1H, H-5'); 2.16 (d, J = 3 c/s, 1H, H-2'); 3.42, 3.48 (s, 2H, H-3, 3"); 3.52 (d, J = 3 c/s, 1H, H-8); 3.66 (d, J = 3 c/s, 1H, H-6); 3.38 (s, 1H, H-6"); 5.94, 6.08 (s, 3H each, OMe-5,5"); 6.12, 6.28 (s, 3H each, OMe-7, 7"); 6.25, 6.27 (s, 3H each, OMe-4',4"').

Biflavonyls from heartwood of Garcinia livingstonii:

Dried and well powdered heartwood (4 Kg) was refluxed with petroleum ether (40-60°; 3 x 8 liter). The combined extracts were concentrated under reduced pressure. An oily residue was left behind which gave a negative cyanidin test.

The defatted heartwood was then extracted with hot

acetone till the extract was almost colourless. The combined extracts were concentrated under diminished pressure whereby a highly viscous dark brown concentrate was left behind. This was refluxed successively with petroleum ether(40-60°), benzene, and chloroform to remove non-flavonoidic constituents. The residue was treated with hot water. The brownish gummy mass was then refluxed with ethyl acetate (1 liter) for 8 hr and filtered. The filtrate was concentrated to 50 ml and ether was added gradually till there was no more precipitation. It was filtered and the precipitate rejected as it gave no test for flavonoids. The filtrate was evaporated to dryness to give a light brown residue (3 g) which showed the usual flavonoidic colour reactions.

Separation of biflavones-Preparative thin-layer chromatography:

Thin-layer chromatographic examination of biflavonyl mixture in seven solvent systems listed earlier indicated the presence of two closely spaced components. This mixture was separated into two components by repeated preparative thin-layer chromatography on silica gel (NCL Poona) using benzene:pyridine:formic acid (36:9:5) as the developing system. The homogeneity of the pigments was checked by TLC.

3"',4',4"',5,5",7,7"-Heptahydroxy-3(8"-) flavonyl-flavanone (BGH-II):

Slowly crystallized from methanol as yellow plates (600 mg), m.p. 300°, R_F 0.023, $[\alpha]_D^{25} \pm 0$ (methanol, 1 mg/ml, λ_{max}^{EtOH} 275, 288 and 345 nm. [Found: C, 60.06; H, 4.17, Calc. for $C_{30}H_{20}O_{11}2H_2O$: C, 60.8; H, 4.05].

2',3"',4,4',4"',5",6',7"-Octaacetyl-α(8"-) flavonyl chalcone:

A mixture of biflavone (100 mg), pyridine (1 ml), and acetic anhydride (1 ml) was refluxed at 95° on a water bath for 2 hr. The reaction mixture was cooled and poured onto crushed ice. The white solid was filtered, washed, and dried. It crystallized from chloroform-methanol to give colourless prisms (70 mg), m.p. 212-15°, ν_{max}^{KBr} 1780, 1660, 1600, 1500, 1380, 1200, 1120, 1090, 1070, 1050, 1020, 900, and 950 cm^{-1} [Found: C, 61.76; H, 3.93. Reqd. for $C_{46}H_{36}O_{19}$: C, 61.9; H, 4.0].

NMR ($CDCl_3$): values on τ scale:

3.01 (d, J = 9 c/s, 2H, H-3,5); 2.47 (d, J = 9 c/s, 2H, H-2,6); 2.15 (q, J_1 = 9 c/s and J_2 = 3 c/s, 1H, H-6"'); 2.10 (d, J = 3 c/s, 1H, H-2"'); 2.68 (d, J = 9 c/s, 1H, H-5"')

3.38 (s, 1H, H-3"); 3.92 (s, 1H, H-β); 3.21 (s, 1H, H-6"); 3.40 (d, J = 3 c/s, 1H, H-3'); 3.52 (d, J = 3 c/s, 1H, H-5'); 7.62 (s, 3H, OAc); 7.69 (s, 3H, OAc); 7.70 (s, 3H, OAc); 7.72 (s, 3H, OAc); 7.78 (s, 6H, 2 OAc); 8.08 (s, 3H, OAc).

3"',4',4"',5,5",7,7"-Hepta-O-methyl-3(8"-) flavonyl flavanone:

The biflavonyl (100 mg), anhydrous potassium carbonate (2 gm), methyl iodide (1 ml) and dry acetone (100 ml) were refluxed for 12 hr with further addition of potassium carbonate (1 g) and methyl iodide (0.5 ml) after 6 hr. The reaction mixture after usual work up yielded a white solid which was purified by preparative TLC. It crystallized from ethyl acetate-petroleum ether (60-80°) as colourless plates (65 mg), m.p. 212-13°, R_F 0.395, λ_{max}^{EtOH} 228, 274, and 336 nm. ν_{max}^{nujol} 1670, 1645, 1620, 1580, 1530, 1360, 1320, 1270, 1220, 1180, 1170, 1150, 1120, 1070, 1050, 1030, 1020, 865, 840, and 770 cm^{-1}. [Found: C, 66.4; H, 5.09; Mol. Wt. 654 (mass) Reqd. for $C_{37}H_{34}O_{11}$: C, 67.9; H, 5.2].

NMR ($CDCl_3$): values on τ scale:

2.91 (d, J = 9 c/s, 2H, H-2',6'); 3.40 (d, J = 9 c/s, 2H, H-3',5'); 2.6 (q, J_1 = 9 c/s, J_2 = 3 c/s, 1H, H-6"'); 2.85 (d, J = 3 c/s, 1H, H-2"'); 3.2 (d, J = 9 c/s, 1H, H-5"');

3.54 (s, 1H, H-3"); 4.16 (d, J = 12 c/s, 1H, H-2); 5.08 (d, J = 12 c/s, 1H, H-3); 3.74 (s, 1H, H-6"); 3.81 (d, J = 3 c/s, 1H, H-8); 3.88 (d, J = 3 c/s, 1H, H-6); 6.10 (s, 3H, OMe); 6.12 (s, 3H, OMe); 6.17 (s, 3H, OMe); 6.19 (s, 6H, 2 OMe); 6.36 (s, 6H, 2 OMe).

Ozonolysis of BGH-II octaacetate:

A stream of ozonised oxygen was passed through a cooled (-20° to -30°) solution of BGH-II octaacetate (60 mg) in dry ethyl acetate (15 ml) until the theoretical amount of ozone was generated. The solution was allowed to attain room temperature (1/2 hr) and was then hydrogenated at atmospheric pressure in the presence of 5% Pd-charcoal (100 mg) until the rapid intake of hydrogen ceased. The catalyst was filtered off and solvent was reduced to 2 ml. The reaction mixture on co-chromatography (silica Gel, E. Merck; Benzene:dioxan:acetic acid, 90:25:4, as developing solvent system) with an authentic sample showed the presence of p-acetoxy benzaldehyde. The shades of spots with p-diazotized sulphanilic acid as chromogenic reagent were also identical.

4',4"',5,5",7,7"-Hexahydroxy-3(8"-) flavonyl flavanone (BGH-III):

Slowly crystallized from methanol as yellow plates

(550 mg), m.p. 267-68°, R_F 0.21, λ_{max}^{EtOH} 278, 290, and 340 nm. [Found: C, 61.19; H, 4.26. Reqd. for $C_{30}H_{20}O_{10} \cdot 3H_2O$: C, 61.2; H, 4.42].

2',4,4',4"',5",6',7"-Heptaacetyl-α (8"-) flavonyl chalcone:

BGH-III (100 mg), pyridine (1.5 ml) and acetic anhydride (1.5 ml) were heated on a water bath for 2 hr. The reaction mixture was cooled to room temperature and poured onto crushed ice. The solid was filtered, washed and dried. It crystallized from chloroform-methanol as colourless needles (78 mg), m.p. 202-05°, ν_{max}^{nujol} 1760, 1640, 1620, 1600, 1500, 1360, 1190, 1170, 1125, 1090, 1065, 1050, 1015, 900 and 850 cm^{-1} [Found: C, 61.19; H, 4.26. Reqd. for $C_{44}H_{34}O_{17} \cdot H_2O$: C, 62.0; H, 4.2].

NMR ($CDCl_3$): values on τ scale:

2.45 (d, J = 9 c/s, 2H, H-2,6); 3.0 (d, J = 9 c/s, 2H, H-3,5); 3.38 (d, J = 2 c/s, 1H, H-3'); 3.49 (d, J = 2 c/s, 1H, H-5'); 3.92 (s, 1H, H-β); 1.98 (d, J = 8 c/s, 2H, H-2"', 6"'); 2.75 (d, J = 8 c/s, 2H, H-3"', 5"'); 3.22 (s, 1H, H-6"); 3.36 (s, 1H, H-3"); 7.62 (s, 3H, OAc); 7.69 (s, 3H, OAc); 7.70 (s, 3H, OAc); 7.72 (s, 3H, OAc); 7.78 (s, 6H, 2 OAc); 8.08 (s, 3H, OAc).

4',4"',5,5",7,7"-Hexa-O-methyl-3(8"-) flavonyl flavanone:

BGH-III (100 mg), methyl iodide (1.5 ml) and potassium carbonate (2 g) were refluxed in dry acetone (100 ml) for 16 hr with further addition of methyl iodide (1 ml) and potassium carbonate (1 g) after 6 hr. After usual work up and purification by preparative thin-layer chromatography, the methyl ether crystallized from chloroform-methanol as colourless cubes. (76 mg), m.p. 254-56°, R_F 0.42, λ_{max}^{EtOH} 228, 284 nm, ν_{max}^{nujol} 1670, 1645, 1620, 1600, 1520, 1340, 1315, 1255, 1230, 1220, 1180, 1165, 1120, 1045, 1030, 830, 815 and 725 cm^{-1}. [Found: C, 68.93; H, 5.42. Mol. Wt. 624 (mass). Reqd. for $C_{36}H_{32}O_{10}$: C, 69.2; H, 5.11].

NMR ($CDCl_3$): values on τ scale:

2.95 (d, J = 9 c/s, 2H, H-2',6'); 3.44 (d, J = 9 c/s, 2H, H-3',5'); 2.4 (d, J = 8 c/s, 2H, H-2"', 6"'); 3.2 (d, J = 8 c/s, 2H, H-3"', 5"'); 4.28 (d, J = 12 c/s, 1H, H-2); 5.18 (d, J = 12 c/s, 1H, H-3); 3.7 (d, J = 2 c/s, 1H, H-8); 3.9 (d, J = 2 c/s, 1H, H-6); 3.82 (s, 1H, H-6"); 3.59 (s, 1H, H-3"); 6.08 (s, 3H, OMe); 6.13 (s, 3H, OMe); 6.17 (s, 6H, 2 OMe); 6.20 (s, 3H, OMe); 6.36 (s, 3H, OMe).

Ozonolysis of BGH-III heptaacetate:

A stream of ozonised oxygen was passed through a cooled (-20° to -30°) solution of BGH-III heptaacetate (40 mg) in dry ethyl acetate (10 ml) for 1/2 hr. The solution was allowed to attain room temperature (1/2 hr) and was then hydrogenated at atmospheric pressure in the presence of 5% Pd-charcoal (100 mg). After the completion of the reaction (2 hr) the catalyst was filtered off and solvent reduced to 2 ml. R_F values and colour shades (with diazotized sulphanilic acid as chromogenic reagent) of the product of the reaction mixture were identical with those of p-acetoxy benzaldehyde.

Extraction of biflavonyls from bark of G. livingstonii:

The defatted powdered bark (2 Kg) was extracted with hot acetone (3 x 6 liter) and the solvent was removed in vacuo. The reddish viscous mass was refluxed successively with petroleum ether (40-60°), benzene, and chloroform and water to remove non-flavonoidic constituents. The brownish gummy residue was then refluxed with ethyl acetate (500 ml) for 8 hr and filtered. The filtrate was concentrated to 50 ml and ether was added gradually till there was no more of precipitation. It was filtered and the precipitate rejected as it gave no colour test for flavonoids. The

filtrate on evaporation yielded a light brown solid (2 g) which showed the flavonoidic colour tests. The brown solid was separated into two homogenous components by repeated preparative thin-layer chromatography (silica gel, NCL, Poona, benzene:pyridine:formic acid, 36:9:5, as developing solvent).

3"',4',4",5,5",7,7"-Heptahydroxy-3(8"-) flavonyl flavanone (BGH-II):

Crystallized from methanol as yellow plates (330 mg), m.p. 300°, R_F 0.02, $[\alpha]_D^{25} \pm 0$ (methanol, 1 mg/ml); λ_{max}^{EtOH} 275, 288, 345 nm, [Found: C, 60.06; H, 4.17. Reqd. for $C_{30}H_{20}O_{11} \cdot 2H_2O$: C, 60.8; H, 4.05].

2',3"',4,4',4"',5",6',7"-Octaacetyl-α (8"-) flavonyl chalcone:

A mixture of BGH-II (100 mg), pyridine (1 ml) and acetic anhydride (1 ml) was refluxed at 95° on a water bath for 2 hr. The reaction mixture was poured onto crushed ice and left over night. The white solid was filtered washed and dried. It crystallized from chloroform-ethanol to give colourless prisms (65 mg), m.p. 212-15°, ν_{max}^{KBr} 1780, 1660, 1600, 1500, 1380, 1200, 1120, 1090, 1070, 1050, 1020, 900 and 850 cm^{-1} [Found: C, 61.76; H, 3.93. Reqd. for $C_{46}H_{36}O_{19}$: C, 61.9; H, 4.00].

NMR ($CDCl_3$): values on τ scale:

3.01 (d, J = 9 c/s, 2H, H-3,5); 2.47 (d, J = 9 c/s, 2H, H-2,6); 2.15 (q, J_1 = 9 c/s and J_2 = 3 c/s, 1H, H-6"'); 2.10 (d, J = 3 c/s, 1H, H-2"'); 2.68 (d, J = 9 c/s, 1H, H-6"'); 3.38 (s, 1H, H-3"); 3.92 (s, 1H, H-β); 3.21 (s, 1H, H-6"); 3.40 (d, J = 3 c/s, 1H, H-3'); 3.52 (d, J = 3 c/s, 1H, H-5'); 7.62 (s, 3H, OAc); 7.69 (s, 3H, OAc); 7.70 (s, 3H, OAc); 7.72 (s, 3H, OAc); 7.72 (s, 3H, OAc); 7.78 (s, 6H, 2 OAc); 8.08 (s, 3H, OAc).

3"',4',4"',5,5",7,7"-Hepta-O-methyl-3 (8"-) flavonyl flavanone:

A mixture of BGH-II (100 mg), anhydrous potassium carbonate (3 g) and methyl iodide (2 ml) in dry acetone (100 ml) was refluxed for 12 hr. The reaction mixture on purification by preparative thin-layer chromatography yielded a white solid which crystallized from ethyl acetate-petroleum ether (40-60°) as colourless plates (60 mg), m.p. 212-13°, R_F 0.39, λ_{max}^{EtOH} 228, 274, 336 nm, ν_{max}^{nujol} 1670, 1645, 1620, 1580, 1530, 1360, 1320, 1270, 1220, 1180, 1170, 1150, 1120, 1070, 1050, 1030, 1020, 865, 840, 830 and 770 cm^{-1} [Found: C, 66.4; H, 5.09. Mol. Wt. 654 (mass). Reqd. for $C_{37}H_{34}O_{11}$: C, 67.9; H, 5.2%].

NMR ($CDCl_3$): values on τ scale:

2.91 (d, J = 9 c/s, 2H, H-2',6'); 3.40 (d, J = 9 c/s, 2H, H-3',5'); 2.6 (q, J_1 = 9 c/s and J_2 = 3 c/s, 1H, H-6"'); 2.85 (d, J = 3 c/s, 1H, H-2"'); 3.2 (d, J = 9 c/s, 1H, H-5"'); 3.54 (s, 1H, H-3"); 4.16 (d, J = 12 c/s, 1H, H-2); 5.08 (d, J = 12 c/s, 1H, H-3); 3.74 (s, 1H, H-6"); 3.81 (d, J = 3 c/s, 1H, H-8); 3.88 (d, J = 3 c/s, 1H, H-6); 6.10 (s, 3H, OMe); 6.12 (s, 3H, OMe); 6.17 (s, 3H, OMe); 6.19 (s, 6H, 2 OMe); 6.36 (s, 6H, 2 OMe).

4',4"',5,5",7,7"-Hexahydroxy-3(8"-) flavonyl flavanone (BGH-III):

Slowly crystallized from methanol as yellow plates (250 mg), m.p. 267-68°, R_F 0.211, λ_{max}^{EtOH} 278 (infl.), 290, 340 nm. [Found: C, 61.19; H, 4.26. Calc. for $C_{30}H_{20}O_{10} \cdot 3H_2O$: C, 61.2; H, 4.42].

2',4,4',4"',5",6',7"-Heptaacetyl-α(8"-) flavonyl chalcone:

A mixture of BGH-III (70 mg), pyridine (1 ml) and acetic anhydride (1 ml) was heated on a boiling water bath for 2 hr. The reaction mixture was poured onto crushed ice. The solid was filtered, washed and dried. It crystallized from chloroform-methanol as colourless needles (45 mg), m.p. 202-05°, ν_{max}^{nujol} 1760, 1640, 1620, 1600, 1500, 1360,

1190, 1170, 1125, 1090, 1065, 1050, 1015, 900 and 850 cm^{-1}. [Found: C, 61.19; H, 4.26. Reqd. for $C_{44}H_{34}O_{17}.H_2O$: - C, 62.0; H, 4.2].

NMR ($CDCl_3$): values on τ scale:

2.45 (d, J = 9 c/s, 2H, H-2,6); 3.0 (d, J = 9 c/s, 2H, H-3,5); 3.38 (d, J = 2 c/s, 1H, H-3'); 3.49 (d, J = 2 c/s, 1H, H-5'); 3.92 (s, 1H, H-β); 1.98 (d, J = 8 c/s, 2H, H-2"', 6"'); 2.75 (d, J = 8 c/s, 2H, H-3"',5"'); 3.22 (s, 1H, H-6"); 3.36 (s, 1H, H-3"); 7.62 (s, 3H, OAc); 7.69 (s, 3H, OAc); 7.70 (s, 3H, OAc); 7.72 (s, 3H, OAc); 7.78 (s, 6H, 2 OAc); 8.08 (s, 3H, OAc).

4',4"',5,5",7,7"-Hexa-O-methyl-3(8"-) flavonyl flavanone:

BGH-III (60 mg), methyl iodide (2 ml) and anhydrous potassium carbonate (3 g) were refluxed in dry acetone (100 ml) for 16 hr. After usual work up of the reaction mixture and purification by preparative thin-layer chromatography, the methyl ether crystallized from chloroform-methanol as colourless cubes (40 mg), m.p. 254-56°, R_F 0.42, λ_{max}^{EtOH} 228, 284 nm, ν_{max}^{nujol} 1670, 1645, 1620, 1600, 1520, 1340, 1315, 1255, 1230, 1220, 1180, 1165, 1120, 1045, 1030, 830, 815 and 795 cm^{-1}. [Found: C, 68.93; H, 5.42. Mol. Wt. 624 (mass). Reqd. for $C_{36}H_{32}O_{10}$: C, 69.2; H, 5.11].

NMR ($CDCl_3$): values on τ scale:

2.95 (d, J = 9 c/s, 2H, H-2',6'); 3.44 (d, J = 9 c/s, 2H, H-3',5'); 2.44 (d, J = 8 c/s, 2H, H-2"',6"'); 3.22 (d, J = 8 c/s, 2H, H-3"',5"'); 4.28 (d, J = 12 c/s, 1H, H-2); 5.18 (d, J = 12 c/s, 1H, H-3); 3.7 (d, J = 2 c/s, 1H, H-8); 3.9 (d, J = 2 c/s, 1H, H-6); 3.82 (s, 1H, H-6"); 3.59 (s, 1H, H-3"); 6.08 (s, 3H, OMe); 6.13 (s, 3H, OMe); 6.17 (s, 3H, OMe); 6.20 (s, 3H, OMe); 6.36 (s, 3H, OMe).

SYNTHESIS OF A NEW SERIES OF BIFLAVONES

3"',4',4"',5,5",7,7"-Hepta-O-methyl-3,8"-biflavone (WGH-III heptamethyl ether):

To a solution of BGH-II (200 mg) in glacial acetic acid (2 ml) was added freshly fused potassium acetate (400 mg). Iodine (200 mg) in glacial acetic acid (2 ml) was added to the boiling mixture slowly during 1 hr and refluxing continued for another hour. The reaction mixture was cooled to room temperature (1/2 hr) and poured into ice cold water containing some crystals of sodium thiosulphate. The yellow precipitate obtained was filtered, washed and dried. The yellow solid (180 mg) was methylated with methyl iodide (2 ml) and potassium carbonate (2 g) in acetone (100 cc). After purification by preparative thin-layer chromatography, the methyl ether crystallized from chloroform-methanol as colourless

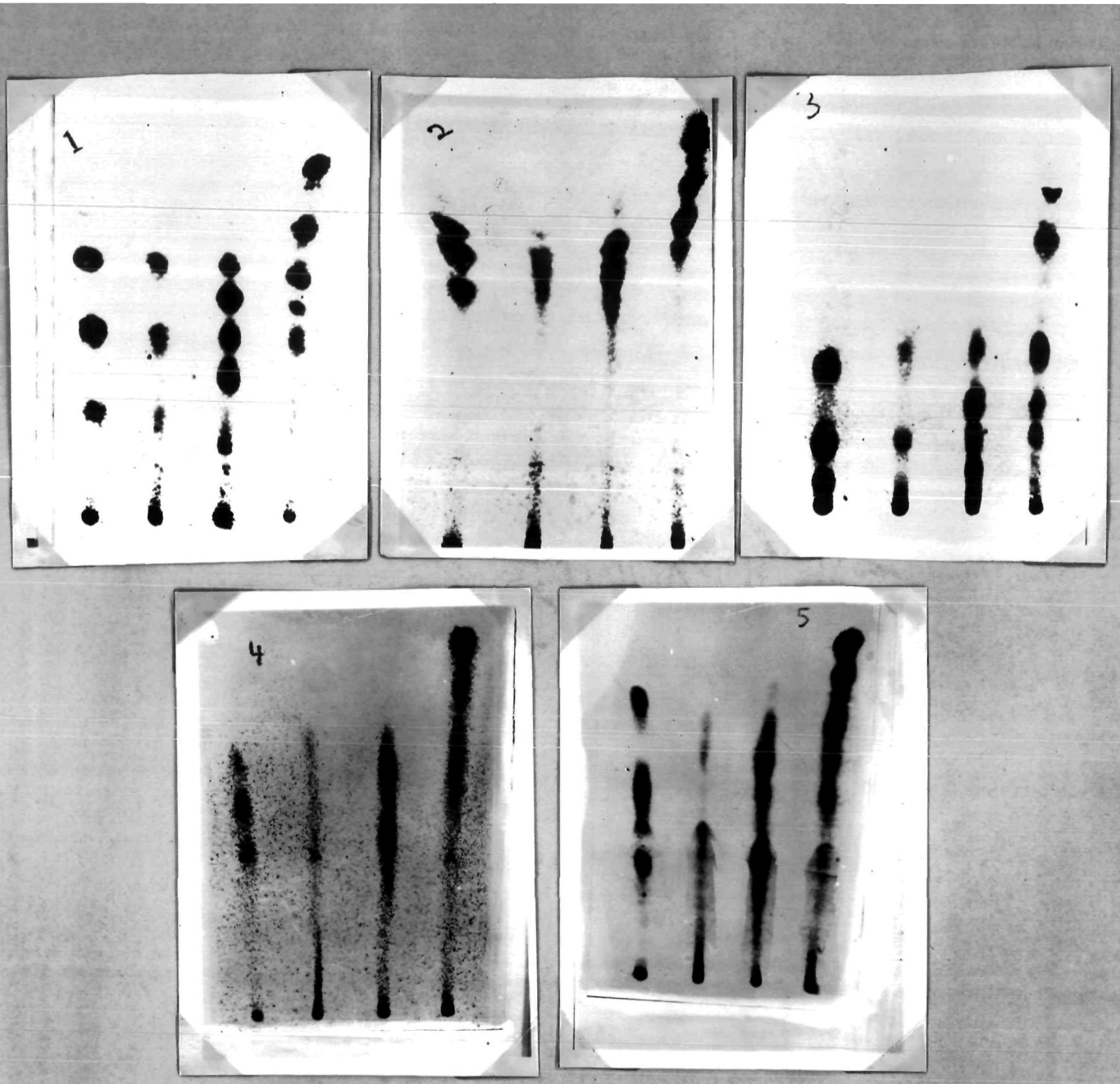


Fig. VIII Chromatograms of biflavonyls from leaf extracts of (left to right) Podocarpus gracilior Pilger, Cryptomeria japonica, Agathis palmerstronii and Araucaria cookii in solvent systems:

1. benzene-pyridine-formic acid (36:9:5); 2. toluene-ethyl formate-formic acid (5:4:1); 3. toluene-pyridine-acetic acid (10:1:1); 4. benzene-pyridine-ethyl formate-dioxan (5:1:2:2); 5. benzene-ethyl acetate-acetic acid (8:5:2).

needles (110 mg), m.p. 150-52°, R_F 0.37, [Found: C, 67.4; H, 5.01, Mol. Wt. 652 (mass). Reqd. for $C_{37}H_{32}O_{11}$: C, 67.2; H, 5.0]7.

NMR ($CDCl_3$): values on τ scale:

2.68 (d, J = 9 c/s, 2H, H-2',6'); 3.20 (d, J = 9 c/s, 2H, H-3',5'); 2.74 (q, J_1 = 10 c/s, J_2 = 2 c/s, 1H, H-6"'); 3.32 (d, J = 10 c/s, 1H, H-5"'); 2.94 (d, J = 2 c/s, 1H, H-2"'); 3.49, 3.61 (two singlets, 2H, H-3",6"); 3.44 (d, J = 2 c/s, 1H, H-6); 3.62 (d, J = 2 c/s, 1H, H-8); 6.00 (s, 3H, OMe); 6.10 (s, 3H, OMe); 6.12 (s, 3H, OMe); 6.15 (s, 3H, OMe); 6.25 (s, 3H, OMe); 6.27 (s, 3H, OMe); 6.56 (s, 3H, OMe).

4',4"',5,5",7,7"-Hexa-O-methyl-3,8"-biflavone (WGH-III hexamethyl ether):

To a solution of BGH-III (200 mg) in glacial acetic acid (2 ml) was added properly fused potassium acetate (400 mg). Iodine (200 mg) in glacial acetic acid (2 ml) was added to the boiling mixture slowly during 1 hr and refluxing was continued for another hour. The reaction mixture was cooled to room temperature (1/2 hr) and poured into ice cold 5% solution of sodium thiosulphate. The yellow precipitate (180 mg) was filtered washed and dried. It was refluxed with methyl iodide (1.5 ml) and potassium carbonate

(2 g) in acetone for 16 hr. After usual work up and purification by preparative thin-layer chromatography, the methyl ether crystallized from chloroform-methanol as fine colourless needles (80 mg), m.p. 175°, R_F 0.40. [Found: C, 69.3; H, 4.8. Mol. Wt. 622 (mass). Reqd. for $C_{36}H_{30}O_{10}$: C, 69.45; H, 4.9].

NMR ($CDCl_3$): values on τ scale:

2.67 (d, J = 9 c/s, 2H, H-2',6'); 3.32 (d, J = 9 c/s, 2H, H-3',5'); 2.51 (d, J = 8 c/s, 2H, H-2"',6"'); 3.23 (d, J = 9 c/s, 2H, H-3"',5"'); 3.42 (d, J = 2 c/s, 1H, H-8); 3.60 (d, J = 2 c/s, 1H, H-6); 3.55, 3.62 (2 singlets, 2H, H-3",6"); 6.00 (s, 3H, OMe); 6.09 (s, 3H, OMe); 6.10 (s, 3H, OMe); 6.24 (s, 3H, OMe); 6.26 (s, 3H, OMe); 6.29 (s, 3H, OMe).

THIN-LAYER CHROMATOGRAPHY OF BIFLAVONYLS ON SILICA GEL

All reagents used were of 'ANALAR' grade excepting formic acid (E. Merck) and ethyl formate (Bush & Co.). Using a thin layer applicator (Desaga, Heidelberg) glass plates (20 x 20 cm) were coated with a well-stirred suspension of silica gel G (E. Merck; 50 gm - 95 ml water) to give a layer approximately 0.5 mm in thickness. After drying for 2 hr at room temperature, the plates were activated at 110-120° for 1 hr and preserved in a desiccator until required.

Pure samples of fully methylated biflavonyls (1 mg/ml in $CHCl_3$) and parent and partial methyl ethers (1 mg/ml in pyridine) were applied with suitable micro-liter pipettes at the starting line (2 cm from the lower edge of plate and 2 cm apart from each other). The plates were mounted on a stainless steel frame and placed in Desaga glass chamber 10 x 22 x 21 cm containing 200 ml of solvent. When the solvent front travelled 15 cm from the starting line the development was interrupted and the plates were dried at room temperature. Spots were located either in UV light or by spraying $FeCl_3$-EtOH or diazolized sulphanilic acid as chromogenic reagents.

Preparation of chromogenic reagents:

$FeCl_3$ soln: 1% ethanolic soln. of $FeCl_3$ was used as spray reagent.

Diazotized sulphanilic acid[97]:

Cold mixture of 2N KOH (225 ml) containing 50 g sulphanilic acid and 10% $NaNO_2$ (200 ml), was added dropwise with stirring to a solution of 18N H_2SO_4 (80 ml) and water (40 ml) at 0°. The precipitated p-sulphobenzene-diazonium salt was filtered off, washed successively with ice cold water, ethanol and ether and air dried. The diazotized sulphanilic acid (0.4 g) was dissolved in 100 ml of 2N NaOH and used as spray reagent.

Origin of biflavonyls:

All the biflavonyls (except morelloflavone, GB-1, GB-2 and sciadopitysin) were either isolated or synthesized in our laboratories. The isolation of biflavonyls from Podocarpus gracilior is described as a typical example of the procedure.

A. Isolation of biflavonyls from Podocarpus glacilior:

Dried leaves of P. gracilior were extracted with boiling petroleum ether (60-80°) to remove the waxy and resinous matters. Defatted leaves were completely exhausted with acetone and filtered. The solvent was distilled off and the green residue left behind was treated successively with benzene, chloroform and boiling water. The insoluble mass gave usual colour reactions of flavonoids. It was purified by passing over a column of magnesium silicate (Woelm) using elutropic series of organic solvents. The acetone and ethyl acetate (saturated with water) fractions were combined. The solid obtained was separated into four components by the use of preparative thin-layer chromatography (silica gel; benzene:pyridine:formic acid, 36:9:5 as developing system). They were characterized as amentoflavone, podocarpusflavone A, isoginkgetin and kayaflavone by m.ps., mixed m.ps., and spectral studies of the parent compounds and their derivatives.

Biflavonyls	Source
Amentoflavone, podocarpusflavone A, isoginkgetin and kayaflavone.	Podocarpus gracilior[104]
Bilobtin and ginkgetin	Ginkgo biloba[3]
Sotetsuflavone	Cycas revoluta[3]
Cupressuflavone and isocryptomerin	Cupressus funebris[105]
7,7"-Di-O-methyl cupressuflavone, 4',4"', 7,7"-tetra-O-methyl cupressuflavone and 4',4"', 7,7"-tetra-O-methyl amentoflavone.	Araucaria cookii and A. cunninghamii[40]
Agathisflavone A and Agathisflavone B	Agathis palmerstonii[6]
Agathisflavone	Agathis robusta[105]
7,7"-Di-O-methyl agathisflavone	Agathis alba[106]
7-O-Methyl cupressuflavone	Araucaria bidiwilli[82]
Hinokiflavone (4'-O-6") and cryptomerin B.	Cryptomeria japonica[107]
BGH-II and BGH-III	Garcinia livingstonii.

B. Fully methylated biflavonyls:

Complete methyl ethers of amentoflavone, cupressuflavone, agathisflavone, BGH-II, BGH-III, WGH-II, WGH-III

and hinokiflavone (4'-O-6") were prepared by the general procedure as described below:

Biflavone (100 mg), methyl iodide (2 ml) and anhydrous potassium carbonate (2 g) were refluxed in dry acetone (100 ml) for 10-12 hr with further addition of methyl iodide (1 ml) and anhydrous potassium carbonate (1 g) after 4 hr. The reaction mixture was filtered and the residue washed with hot acetone (6-7 times). The filtrate and washings were combined and the solvent was distilled off, the yellow residue left behind was taken up in chloroform (100 ml) and washed with water in a separatory funnel (6-7 times) to remove traces of potassium carbonate. The chloroform extract was reduced to 20 ml and purified by preparative thin-layer chromatography (silica gel, benzene: pyridine:formic acid, 36:9:5 as developing system).

The compounds were crystallized from suitable solvent mixtures.

C. Synthetic biflavonyls:

(i) 4"',5,5",7,7"-Penta-O-methyl hinokiflavone (4'-O-8"):

It was prepared by modification of Nakazawa's synthesis[42]. A mixture of 4'-Iodo-3-nitro-5,7-dimethoxy flavone (1.4 g) and 8-Hydroxy-4',5,7-trimethoxy flavone (0.8 g), potassium carbonate (1 g) and DMSO (10 ml) was

heated at 110° for 1 hr with occasional stirring. The reaction mixture was poured into water (50 ml), when a dark grey precipitate separated. It was filtered, washed with water and dried. To a stirring suspension of 3-nitro-hinokiflavone pentamethyl ether (4'-O-8") (1 g) in a solution of DMF (40 ml), water (20 ml) and acetic acid (50 ml) was added sodium thiosulphate (2 g) at 80-90° over a period of 20 minutes. After further stirring for 40 minutes, sodium acetate (1 g) was added to the reaction mixture and after cooling for 2 hr the crystals of amine were filtered. A solution of amine (1 g) in DMF (40 ml) and 10% HCl (10 ml) was diazotized with 10% sodium nitrite. After addition of 50% H_3PO_2 (4 ml) the reaction mixture was left overnight. The separated solid was filtered, washed, dried and purified over a column of silica gel using chloroform as eluent. It crystallized from chloroform-methanol as fine needles (120 mg) m.p. 268°.

(ii) 4',7,7"-Tri-O-methyl cupressuflavone[108]:

Cupressuflavone hexamethyl ether (100 mg), hydriodic acid (d, 1.7; 1.5 ml), phenol (0.1 ml) and acetic anhydride (0.3 ml) were heated at 100° for 1 hr. The reaction mixture was poured into ice cold sodium thiosulphate solution (5%, 200 ml). The precipitate was filtered, washed and dried. It was purified by preparative thin-layer chromatography and

was characterized as 4',7,7"-tri-O-methyl cupressuflavone by spectral studies.

(iii) 4,4"',5,5",7,7"-Hexa-O-methyl amentoflavone (3'-6" linked)[108]:

A mixture of amentoflavone hexamethyl ether (200 mg), hydriodic acid (d, 1.7; 5 g) and acetic anhydride (1 ml) was refluxed at 130-140° for 8 hr. The reaction mixture was poured into ice cold sodium thiosulphate solution (5%, 200 ml). The yellow precipitate was filtered, washed with water and dried. The crude reaction mixture was methylated with methyl iodide (2 ml) and anhydrous potassium carbonate (2 g) in dry acetone (100 ml). After usual work up the mixture was separated by preparative thin-layer chromatography into unisomerized (m.p. 227-28°) and isomerized (m.p. 292°) amentoflavone hexamethyl ethers.

(iv) WGH-II and WGH-III:

Both the biflavones were synthesized as described earlier.

D. Samples of morelloflavone, GB-1, GB-2 and sciadopitysin were obtained as generous gifts from Dr. K. Venkataraman, B. Jackson and N. Kawano respectively.

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